Physiological and Behavioural Flexibility in African Striped Mice: Testosterone and Environmental Influences

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Content

Summary

Flexibility – reversible phenotypic changes in physiology, morphology, and / or behaviour – has been extensively studied in the framework of alternative reproductive tactics (ARTs). A reproductive tactic is a phenotype that results from a strategy – a decision rule based on genetic program. The relative plasticity hypothesis states that environmentally-induced hormonal changes cause the development of one out several possible ARTs. However, causality between hormones and ARTs has been poorly studied. Further, it is important to determine which environmental factors influence hormonal changes in males of different ARTs to determine in how far these hormonal changes are flexible.

In this thesis, I experimentally studied the role of testosterone in causing physiological, morphological, and behavioural differences between two alternative reproductive tactics in male African striped mice, *Rhabdomys pumilio*: 1) group-living helpers showing alloparental care, low testosterone levels, and high corticosterone levels. 2) solitary-living roamers showing no parental care, high testosterone levels, and low corticosterone levels. Finally, I tested the flexibility of prolactin secretion as prolactin may play a role in the regulation of ARTs. For this, I studied the role of photoperiod and food availability in regulating prolactin levels of paternal dominant breeding males.

In chapter 1 I studied which factors correlate with alloparental care. For the first time I demonstrated that both male and female, juvenile and adult philopatrics show extensive helping behaviour (14.7 % of their time). Corticosterone levels correlated with alloparental care in an age- and sex-dependent manner. Natural variation of testosterone levels between helpers did not correlate with alloparental care, suggesting that changes in alloparental care after the tactic switch are not caused by testosterone.

In chapter 2, I tested whether an experimental increase of testosterone in male group living helpers influenced alloparental care and dispersal-like behaviours. An experimental increase of testosterone in male group-living helpers did not reduce alloparental care nor aggressive behaviour, but increased boldness, decreased anxiety, and lowered basal corticosterone levels. In the field, exogenous testosterone did not cause dispersal, but it caused male group-living helpers to expand their home ranges (chapter 3). I suggested that an increase of testosterone facilitate dispersal by reducing stress reactivity (low corticosterone levels and low anxiety). Exogenous testosterone also induced sexual maturation and spermatogenesis – testosterone-treated male group-living helpers became scrotal and showed larger testes and epididymis (chapter 3). Thus, I suggested that a quick increase of testosterone levels is an important event during the tactic switch to cause necessary

physiological, morphological, and behavioural changes in group-living helpers to disperse and become solitary-living roamers.

In chapter 4 I showed that free-ranging paternal dominant breeding males showed higher prolactin levels when they were breeding compared to the non-breeding season, independently whether this was during spring (increase of photoperiod) or during summer, i.e. normal non-breeding season (decrease of photoperiod). Food availability correlated with prolactin levels, suggesting that cues related to food availability may regulate prolactin levels. As paternal dominant breeding males have higher reproductive success than males following an alternative reproductive tactic (i.e. group-living helpers and solitary-living roamers), flexibility in prolactin secretion seems adaptive.

My thesis demonstrated that testosterone plays an important role in physiological, morphological and behavioural differences between males of different ARTs in African striped mice. I also showed that the role of environmental factors (e.g. food availability) is crucial in hormonal flexibility (prolactin levels). Thus, studies from the African striped mouse suggest a complex relationship between hormonal and environmental factors in the regulation of ARTs. For future studies, I discussed how to integrate environmental factors in the behavioural endocrinology approach to study proximate mechanisms of ARTs.

Zusammenfassung

Flexibilität - reversible phänotypische Veränderungen in Physiologie, Morphologie und/oder Verhalten - wurde ausführlich im Zusammenhang mit alternativen Fortpflanzungsstrategien untersucht. Unter einer Fortpflanzungsstrategie versteht man den Phänotyp der auf eine Strategie zurückzuführen ist – eine Entscheidungsregel basierend auf einem genetischen Programm. Die Relative Plastizitäts-Hypothese besagt, dass hormonelle Änderungen, welche durch die Umwelt induziert werden, die Entwicklung von einer von vielen möglichen alternativen Fortpflanzungsstrategien bewirken. Jedoch wurde der Kausalzusammenhang zwischen Hormonen und alternativen Fortpflanzungsstrategien bisher nur ungenügend untersucht. Zudem ist es wichtig zu bestimmen, welche Umweltfaktoren hormonelle Schwankungen, in Männchen mit unterschiedlichen alternativen Fortpflanzungsstrategien, auslösen. Auf diese Weise kann man herausfinden in wie weit hormonelle Veränderungen flexibel sind.

In dieser Doktorarbeit untersuchte ich experimentell den Einfluss von Testosteron auf physiologische-, morphologische- und Verhaltensunterschiede zwischen zwei alternativen Fortpflanzungsstrategien bei Afrikanischen Striemengrasmäusen, *Rhabdomys pumilio*: 1) Männliche gruppenlebende Helfer zeigen alloparentale Fürsorge, tiefe Testosteronwerte und hohe Kortikosteronwerte. 2) Solitär lebende Roamer zeigen keine elterliche Fürsorge, hohe Testosteronwerte und tiefe Korikosteronwerte. Des Weiteren testete ich die Flexibilität der Prolaktinausschüttung, da Prolaktin vielleicht eine Rolle bei der Regulation der alternativen Fortpflanzungsstrategien spielt. Zu diesem Zweck untersuchte ich den Einfluss der Photoperiode und Futter-Verfügbarkeit auf die Regulierung des Prolaktinspiegels von paternalen dominanten Zuchtmännchen.

Im ersten Kapitel untersuchte ich, welche Faktoren mit alloparentaler Fürsorge korrelieren. Als Erster konnte ich demonstrieren, dass sowohl juvenile wie auch adulte philopatrische Männchen und Weibchen ausgeprägtes Helferverhalten zeigten (14.7% ihrer Zeit). Die Kortikosteronwerte korrelierten mit alloparentaler Fürsorge in Abhängigkeit von Alter und Geschlecht. Natürliche Schwankungen der Testosteronwerte zwischen Helfern korrelierten nicht mit alloparentaler Fürsorge. Dies lässt vermuten, dass Änderungen in der alloparentalen Fürsorge nach einem Strategiewechsel nicht durch Testosteron hervorgerufen wird.

Im zweiten Kapitel testete ich, ob eine experimentelle Erhöhung des Testosterons, in männlichen gruppenlebenden Helfern, alloparentale Fürsorge und Verhaltensweisen, die mit

Abwanderung im Zusammenhang stehen, beeinflussten. Eine experimentelle Erhöhung des Testosterons in männlichen gruppenlebenden Helfern reduzierte weder alloparentale Fürsorge noch aggressives Verhalten. Jedoch erhöhte es Kühnheit, minderte ängstliches Verhalten sowie basale Kortikosteronwerte. Im Feld führte exogenes Testosteron nicht zur Abwanderung, hingegen vergrösserten männliche gruppenlebende Helfer ihre Territorien (Kapitel 3). Ich behauptete, dass eine Erhöhung des Testosterons die Abwanderung erleichtert, indem das Reaktionsvermögen auf Stress erniedrigt wird (tiefe Kortikosteronwerte und tiefe Ängstlichkeit). Zudem induzierte exogenes Testosteron sexuelle Reife und Spermatogenese – mit Testosteron behandelte männliche gruppenlebende Helfer hatten sichtbare und grössere Hoden und Nebenhoden (Kapitel 3). Ich nehme an, dass eine schnelle Erhöhung der Testosteronwerte ein wichtiges Ereignis während eines Strategiewechsels darstellt, um in gruppenlebenden Helfern nötige physiologische-, morphologische- und Verhaltensänderungen hervorzurufen, damit sie abwandern und zu solitären Roamer werden.

Im Kapitel 4 demonstrierte ich, dass freilebende paternale dominante Zuchtmännchen höhere Prolaktinwerte zeigten, wenn sie sich fortpflanzten als ausserhalb der Fortpflanzungszeit. Dies war unabhängig davon, ob es während des Frühlings (Verlängerung der Photoperiode) oder des Sommers, also normalerweise ausserhalb der Fortpflanzungszeit (Verkürzung der Photoperiode), stattfand. Zudem korrelierte Futter-Verfügbarkeit mit den Prolaktinwerten. Dies lässt vermuten, dass Hinweise, die im Zusammenhang mit Futter-Verfügbarkeit stehen, die Prolaktinwerte regulieren. Da paternale dominante Zuchtmännchen einen höheren Fortpflanzungserfolg haben als Männchen, die eine andere Fortpflanzungsstrategie verfolgen (wie zum Beispiel gruppenlebende Helfer und solitär lebende Roamer), scheint eine Flexibilität in der Prolaktinausschüttung adaptiv zu sein.

Meine Doktorarbeit veranschaulicht, dass Testosteron eine zentrale Rolle spielt in physiologischen-, morphologischen- und Verhaltensunterschieden zwischen Männchen mit unterschiedlichen alternativen Fortpflanzungsstrategien bei Afrikanischen Striemengrasmäusen. Ausserdem zeigte ich, dass die Wirkung von Umweltfaktoren (wie zum Beispiel Futter-Verfügbarkeit) ausschlaggebend ist für die Hormonflexibilität (Prolaktinspiegel). Demzufolge deuten Studien von Afrikanischen Striemengrasmäusen auf eine komplexe Beziehung zwischen hormonellen- und Umweltfaktoren, in der Regulierung von alternativen Reproduktionsstrategien, hin. Für zukünftige Studien erörterte ich, wie man Umweltfaktoren in den Verhaltens- und hormonellen Ansatz integrieren könnte, um proximate Mechanismen der alternativen Fortpflanzungsstrategien zu untersuchen.

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General Introduction

General Introduction

Being flexible can be of great advantages to cope with changing environments (Lott, 1991). Phenotypic flexibility covers reversible phenotypic changes in physiology, morphology, and behaviour when environmental conditions change during an organism's lifespan (Piersma & Drent 2003). With the ability of flexibility, organisms do not end up with a permanent phenotype but can adapt to new environments in a way that they can optimize individual fitness by increasing survival and by optimizing their reproductive success (Proppe et al. 2011; Schradin & Lindholm 2011; Schradin et al. 2012b). The efficiency to quickly adjust the phenotype to environmental conditions (Starck 1999; Wikelski & Thom 2000) is thought to rely on evolved physiological mechanisms, for example, environmentally induced hormonal responses (Wingfield et al. 1990; Ketterson & Nolan 1999; Hau 2007; Ketterson et al. 2009; Schradin et al. 2012b). From an eco-physiological perspective, it is important to understand which environmental factors influence physiological traits to determine in how far these traits are flexible. From a behavioural endocrinologist perspective, the question is all about the causal relationship between hormone and behavioural flexibility – an old issue in the study of hormones / behaviour relationships (Nelson 2005). Thus, if one integrates these two perspectives, one may reveal the proximate mechanisms (physiology) leading to flexibility and understand its adaptive value (Wingfield et al. 2008).

Environmental Factors Influencing Physiological Flexibility

Behavioural and physiological flexibilities are essential for seasonal breeding species (Piersma & Drent 2003; Wingfield 2005). Seasonal changes can coincide with photoperiod – changes in day length – and typically time physiological and behavioural changes related to reproduction (Cockrem 1995; Wikelski et al. 2000; Hau 2001; Sharp 2005; Dawson 2008). For instance, when day length increases prolactin secretion increases, which activates reproduction such as in golden hamster, *Mesocricetus auratus* (Goldman et al. 1981; Steger et al. 1983). Photoperiod is a reliable indicator for timing optimally breeding because it correlates well with environmental conditions, i.e. food, water, and temperature (Wingfield 2008). However, changes in environmental conditions are not predictable for all species and in such species photoperiod might not indicate when future necessary resources for breeding will be available (Dawson 2008). In these species, changes in environmental factors such as food, water, and temperature may play a more important role in timing reproduction and regulating the necessary behavioural and physiological changes (Dawson 2008). For instance, the zebra finch, *Taeniopygia guttata*, an opportunistic breeding species living in arid habitat,

can increase prolactin levels outside of the typical breeding season in spring, when food and water availability favours reproduction during other periods of the year (Christensen & Vleck 2008). Similarly in California mice, *Peromyscus californicus*, experimental changes in day length and food availability did not influence prolactin levels but water availability did (Nelson et al. 1995). Such empirical and experimental studies are rare (Nelson et al. 1995; Hau et al. 2004; Christensen & Vleck 2008; Porlier et al. 2012; Watts & Hahn 2012) and in most opportunistically breeding species it is still unclear which environmental factors regulate behavioural and physiological changes.

Alternative Reproductive Tactics

Physiological and behavioural flexibilities have been extensively studied in the framework of alternative reproductive tactics (ARTs). A reproductive tactic is basically a phenotype that results from a strategy, and a strategy is a decision rule – it is usually a genetically based program (Gross 1996). The phenotype resulting from the strategy is selected to optimize the individual fitness (Oliveira et al. 2008). Importantly, the set of behavioural, physiological, and morphological traits of ARTs show discontinuity – the variation of a given trait among ARTs is not a continuum (Oliveira et al. 2008). One can take advantage of ARTs to study same-sex individual differences from a proximate perspective (physiology) (Oliveira et al. 2008).

The Relative Plasticity Hypothesis to Frame Proximate Causes of ARTs

Moore (1991) developed the relative plasticity hypothesis (RPH) by applying ground breaking studies from the late fifties elucidating for the first time the role of steroid hormones in sex determination and differentiation at different life history stages in guinea pig, *Cavia porcellus* (Phoenix et al. 1959) to his studies on ARTs in tree lizards (Moore 1987; Moore & Marler 1987; Moore 1988; Moore & Marler 1988; Thompson & Moore 1989; Moore & Thompson 1990) . Early in life, steroid hormones organize the brain structures and the gonads, determining the sex of most vertebrates - so called sexual differentiation. Later in life, these same hormones activate sexual maturation and sexual behaviours. If this classic model based on organisation and activation actions of hormones can explain sex differences (Phoenix et al. 1959), Moore (1991) thought that this might also explain behavioural differences within a single sex. In analogy with this classic model of sexual differentiation, the RPH relies on the two main types of influences of steroid hormones (Moore 1991): organizational and activational effects (Phoenix et al. 1959). At early life stages, steroid hormones are predicted to determine the reproductive tactics of individuals for the rest of their

life – Moore (1991) defined them as fixed ARTs. In tree lizards, *Urosaurus ornatus*, at early life stages (i.e. at hatching and at 30 days after hatching), they demonstrated that different testosterone exposure lead to different male reproductive tactics (Hews et al. 1994; Hews & Moore 1996). Since this study, the organisational action of steroid hormones in fixed ARTs has been reported, or even better, experimentally demonstrated in several species of fish, amphibians, reptiles, birds, and mammals - for a complete list of studies see (Oliveira et al. 2008). For species that can change reproductive tactics at later life stages, Moore (1991) predicted that the activational influences of steroid hormones are the proximate mechanism regulating the switch from one ART to another one – in opposition to fixed ARTs, he named them plastic ARTs. Importantly, Moore (1991) suggested two main predictions to investigate the RPH: the first prediction involves that steroid hormone levels of species showing fixed ARTs should differ between individuals of different reproductive tactics at early life stages only, whereas, steroid hormone level differences of species showing plastic ARTs should be visible at later life stages only. The second prediction is that the effects of steroid hormones on ART determination should be only effective at early life stages in species showing fixed ARTs and at later life stages in species showing plastic ARTs.

Weaknesses and Strengths of the Relative Plasticity Hypothesis

The number of studies testing the two predictions of RPH is in inverse proportion to their importance. In other words, many studies reported correlates between ARTs and hormonal profile supporting the first prediction of the RPH - Oliveira et al (2008) listed 82 correlative studies among vertebrate species – whereas experimental evidences testing the second prediction are rare – Oliveira et al (2008) listed only 12 experimental hormone manipulation studies. More experimental studies are needed to generalize the RPH to most of vertebrate species showing ARTs. Furthermore, to my knowledge, the RPH has not been explored in any mammalian species. This lack of knowledge between the RPH and mammalian species underlies the need to find an appropriate study organism where such experiments could be conducted.

Although previous studies provided important insight into the relationship between hormones and ARTs (Oliveira et al. 2008), most of these studies did not consider potential hormone interactions. This is a typical problem for experimental hormone manipulation studies. Many authors reported that experimental testosterone manipulations influenced reproductive behaviour, and this was interpreted as a direct causal relationship (Nelson 2005). However, exogenous testosterone can influence other hormone levels, for instance, the down

regulation of stress hormone levels by testosterone and its metabolites (Kitay 1963; Handa et al. 1994; Viau & Meaney 1996; Viau 2002; Seale et al. 2004; Rubinow et al. 2005; Lund et al. 2006). Thus, manipulating testosterone may influence other hormonal changes while influencing behaviour. In other words, testosterone induced changes in other hormonal levels (e.g. glucocorticoids) may cause the behavioural changes. For instance, exogenous testosterone influence anxiety-like behaviour – testosterone-treated mice spent more time in anxiogenic areas than mice that received a placebo (Aikey et al. 2002). However, also glucocorticoids secretion can predict the levels of anxiety in mice (Touma et al. 2008). Thus exogenous testosterone may not directly decrease anxiety, but may decrease corticosterone secretion that may in turn decrease anxiety. In sum, different hormones can work in synergy in the regulation of specific behaviour (Nelson 2005)**.** Thus, it is important to report potential hormonal changes when performing experimental manipulation to provide a better picture of the endocrine mechanism at play in behavioural flexibility.

The African Striped Mouse, Rhabdomys pumilio, as a Species to Study ARTs and to Test the RPH

The African striped mouse is an opportunistically breeding species living in an arid habitat. In the Succulent Karoo of South Africa, they typically reproduce during the spring (August - December) but not during the summer (January - May) and the cold and rainy winter (June and July) (Schradin 2005). This species can also reproduce during the summer after exceptional rainfalls that increase food availability and allow mice to reproduce (Schradin, unpublished data).

The African striped mouse is socially flexible: both males and females can change their reproductive tactics as a function of environmental conditions (Schradin et al. 2012b). While female African striped mouse show clear ARTs and can change from solitary to communal breeding (Schradin et al. 2010) most published studies are about male ARTs (Schradin et al. 2012b). Males can choose among three ARTs which differ in hormone levels and reproductive success (Schradin 2008; Schradin et al. 2009b; Schradin et al. 2009a; Schradin & Lindholm 2011): (i) philopatric group-living helpers with low testosterone levels, high corticosterone levels, and low prolactin levels, have the lowest reproductive success; (ii) solitary-living roamers with high testosterone, low corticosterone levels, and low prolactin levels, have an intermediate reproductive success; (iii) dominant group-living territorial breeders with intermediate testosterone levels, low corticosterone, and high prolactin levels, have the highest reproductive success. To choose among these three ARTs, it seems that

males are following a single strategy based on one set of decision rules (Schradin & Lindholm 2011; Schradin et al. 2012b). Juvenile males that reach the age of sexual maturation (4 weeks old) can either disperse from their natal group in order to choose the roaming tactic, or they can remain in their natal group and choose the helper tactic (Schoepf & Schradin 2012b). Juvenile and philopatric males make the decision to disperse if their body mass is above the population mean and if there are more solitarily-breeding females than communally-breeding females (Schradin & Lindholm 2011). To become a dominant group-living territorial breeder, males have to immigrate into another group of communally breeding females; males cannot become the breeders of their own group. Importantly, males can only become dominant group-living territorial breeders if their body mass is above the mean of the male population, which is not the case in young adult males as body mass is positively correlated with age (Schradin et al. 2009b; Schradin & Lindholm 2011). Two ecological factors can influence the decision to disperse or to remain in the natal group: 1. reproductive competition and 2. population density (Schradin et al. 2010). The former favours dispersal and solitary-living because both males and females will avoid the cost of group-living (Schoepf & Schradin 2012a): males remaining in their natal group as philopatric are typically reproductively suppressed (Schradin et al. 2009b; Schradin et al. 2009a; Schoepf & Schradin 2012b; Schradin et al. 2012a) and adult females reproducing communally face the risk of infanticide (Schradin et al. 2010). Finally, high population density constrains individuals to live in group (Schradin et al. 2010), whereas low population density reduces dispersal costs and thus promotes solitary-living (Schradin et al. 2010; Schoepf & Schradin 2012b).

The hormonal profiles of these three male ARTs fit with the first prediction of the RPH: adult males of different ARTs differ in hormone levels (Schradin 2008; Schradin et al. 2009b). The striped mouse is also the first species where it was demonstrated that individual males change their hormonal profile after they have changed their tactic (Schradin & Yuen 2011). The ARTs are plastic in the term of Moore: males can start initially as philopatric helpers and disperse to become either a roamer or a dominant territorial breeder later in life; a roamer can also choose to become a dominant breeder (Schradin et al. 2009b; Schradin & Lindholm 2011). The hormonal profiles of these males change as soon as the males change reproductive tactics (Schradin & Yuen 2011). For instance, males passing from the roaming to the dominant territorial tactics show a significant decrease in testosterone levels and a significant increase in prolactin levels (Schradin & Yuen 2011). This raises a fundamental question from the RPH perspective, i.e. the second prediction of the RPH: do changes in hormonal levels cause males to change ARTs?

Behavioural changes are observed during tactic switches. Solitary-living roamers do not show alloparental care while philopatric helpers do so (Schradin & Pillay 2004), suggesting that factors influencing alloparental care might be important during the tactic switch. However, we do not know whether age, sex, and the hormonal profiles of helpers influence alloparental care in African striped mice. Hormonal differences in testosterone and corticosterone levels may correlate with differences in alloparental care such as in Mongolian gerbils, *Meriones unguiculatus* (Clark et al. 1998; Clark & Galef 2000). If so, these hormonal-related alloparental care differences might also be due to age or sex differences. Other behavioural and physiological changes are observed during tactic switches. Males which dispersed and became solitary-living roamers were sexually active, i.e. scrotal (Schoepf & Schradin 2012b) and more aggressive towards other males than philopatric helpers which were sexually suppressed, i.e. non-scrotal (Schoepf & Schradin 2012a). Interestingly, dispersing males show higher testosterone levels than non-dispersing males, i.e. males remaining in their natal group as philopatric helpers (Schoepf and Schradin, under review). Testosterone is known to influence parental care, aggressive behaviour (Wingfield et al. 1990) and male reproductive physiology, i.e. steroidogenesis and spermatogenesis. Taken into consideration these pleiotropic effects of testosterone, it is tempting to hypothesize that an increase in testosterone levels enhances the behavioural, morphological, and physiological changes leading to reproductive tactic switches in African striped mice.

Outline of the Thesis

In the chapters 1, 2, and 3, I studied the role of testosterone in the regulation of behavioural, morphological, and physiological flexibility that is observed when philopatric males disperse and become solitary roamers.

In chapter 1 I studied factors that may influence alloparental care in juvenile and adult philopatric helpers. I tested whether age, sex, and testosterone and corticosterone levels influence alloparental care. For this, I observed alloparental care (time spent in the nest with pups, huddling pups, and licking pups) and measured testosterone and corticosterone levels in philopatric helpers of different age and of both sexes: juvenile male helpers, juvenile female helpers, adult male helpers, and adult female helpers. I found that both juvenile and adult male helpers showed significantly higher testosterone levels than adult female helpers. Adult male helpers also showed significantly lower corticosterone levels than the three other types of helpers. Age, sex, corticosterone levels, and their interactions influenced significantly the time spent huddling the pups but not licking the pups. Age, corticosterone levels, and their

interactions tended to influence significantly the time spent in the nest with the pups. Testosterone levels did not influence significantly alloparental care. Ultimately, I discussed these results in term of male-biased dispersal. Proximately, I hypothesized that different mechanisms may operate in alloparental care between male and female helpers.

In chapter 2 I asked whether the difference in testosterone levels between philopatric males and roamers can explain the observed behavioural and physiological differences. For this I manipulated testosterone levels under standard captive conditions. I tested if an increase of testosterone levels decreases corticosterone levels, decreases the expression of alloparental care and aggression, and increases behaviours which are thought to facilitate dispersal (i.e. activity, exploration, boldness, and anxiety-like behaviour) in captive male philopatric helpers. In the same family unit, one male received a testosterone implant and its same-litter brother received a placebo. I found that an increase of testosterone levels did not significantly change the expression of alloparental care and aggression. However, the testosterone treatment increased boldness, activity and decreased anxiety and corticosterone levels. These results demonstrate that testosterone is part of the process involved in the behavioural and physiological flexibility in African striped mice. This also suggests that testosterone may play a role in dispersal and in the regulation of ARTs accordingly to the RPH. I discuss the insensitivity of alloparental care and aggressive behaviour to testosterone in terms of possible side effects of the testosterone treatment itself due to the supra-physiological levels induced by our testosterone treatment.

In chapter 3 I studied the effects of an experimental increase of testosterone levels in free ranging philopatric males in the field in South Africa. I tested whether testosterone implants facilitated juvenile males at the age of puberty (4 weeks old) to increase their home ranges, to disperse, and finally to become solitary roamers. I also tested if an increase of testosterone levels decreases corticosterone levels and enhances sexual maturation, that is, increases gonadal activity measured as the ploidy states of testis cells. I built silastic testosterone implants that increased testosterone levels up to the highest physiological testosterone levels observed in African striped mice. Testosterone-treated males (i.e. males that received testosterone implants) showed larger home ranges than control-treated males (i.e. males that received empty implants). Testosterone-treated males were sexually mature and showed lower corticosterone levels than control-treated males. The testosterone treatment also enhanced spermatogenesis. However, males did not disperse and did not become roamers. I discuss the absence of testosterone-induced dispersal in terms of environmental conditions that did not favour dispersal. In accordance with the results of the chapter 2, these

results demonstrate that an increase of testosterone levels induces behavioural, morphological, and physiological changes that are also observed when males are changing tactics. In other words, testosterone plays an important role in regulating the flexibility observed in African striped mice.

In chapter 4 I tested the influence of environmental factors on prolactin secretion in dominant territorial males: food availability *versus* photoperiod. I compared the prolactin levels in dominant territorial males between spring (day lengths increase and males typically reproduce), one summer without reproduction (day lengths decrease), and one summer with reproduction (day lengths decrease). I demonstrated that males show increased prolactin levels when they were reproducing, that is both during spring and during the summer with reproduction. This study shows that prolactin is regulated independently of photoperiod in striped mice. Furthermore, I show that the prolactin levels positively correlated with food availability. Here, I conclude that the flexibility in prolactin secretion of dominant territorial males is under the control of environmental cues, likely food availability, that indicate adequate environmental conditions for reproduction.

In the general discussion, I sum up my main results and discuss them with regards to the RPH. I present a speculative model of the endocrine regulation of ARTs in African striped mice, specifically the development from juvenile or philopatric helper males into roamers. I finally encourage future experimental studies to integrate environmental factors in the study of endocrine mechanisms of ARTs. For this, I present a protocol – manipulation of both hormonal and environmental factors in a way predicted by my results and previous studies – that may elucidate the proximate mechanism of reproductive tactic switch in African striped mice.

References

Aikey, J. L., Nyby, J. G., Anmuth, D. M. & James, P. J. 2002. Testosterone rapidly reduces anxiety in male house mice (Mus musculus). *Hormones and Behavior*, **42**, 448-460.

Christensen, D. & Vleck, C. M. 2008. Prolactin release and response to vasoactive intestinal peptide in an opportunistic breeder, the zebra finch (Taeniopygia guttata). *General and Comparative Endocrinology*, **157**, 91-98.

Clark, M. M. & Galef, B. G. 2000. Why some male Mongolian gerbils may help at the nest: testosterone, asexuality and alloparenting. *Animal Behaviour*, **59**, 801-806.

Clark, M. M., Vonk, J. M. & Galef, B. G. 1998. Intrauterine position, parenting, and nest-site attachment in male Mongolian gerbils. *Developmental Psychobiology*, **32**, 177-181.

Cockrem, J. F. 1995. Timing of seasonal breeding in birds, with particular reference to new-zealand birds. *Reproduction Fertility and Development*, **7**, 1-19.

Dawson, A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 1621-1633.

Goldman, B. D., Matt, K. S., Roychoudhury, P. & Stetson, M. H. 1981. Prolactin-release in golden-hamsters - photoperiod and gonadal influences. *Biology of Reproduction*, **24**, 287-292.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: Diversity within sexes. *Trends in Ecology & Evolution*, **11**, 92-98.

Handa, R. J., Nunley, K. M., Lorens, S. A., Louie, J. P., McGivern, R. F. & Bollnow, M. R. 1994. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male-rat following novelty and foot shock stressors. *Physiology & Behavior*, **55**, 117-124.

Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *Bioessays*, **29**, 133-144.

Hau, M. 2001. Timing of breeding in variable environments: Tropical birds as model systems. *Hormones and Behavior*, **40**, 281-290.

Hau, M., Wikelski, M., Gwinner, H. & Gwinner, E. 2004. Timing of reproduction in a Darwin's finch: temporal opportunism under spatial constraints. *Oikos*, **106**, 489-500.

Hews, D. K. & Moore, M. C. 1996. A critical period for the organization of alternative male phenotypes of tree lizards by exogenous testosterone? *Physiology & Behavior*, **60**, 425-429.

Hews, D. K., Knapp, R. & Moore, M. C. 1994. Early exposure to androgens affects adult expression of alternative male types in tree lizards. *Hormones and Behavior*, **28**, 96-115.

Ketterson, E. D. & Nolan, V. 1999. Adaptation, exaptation, and constraint: A hormonal perspective. *American Naturalist*, **154**, S4-S25.

Ketterson, E. D., Atwell, J. W. & McGlothlin, J. W. 2009. Phenotypic integration and independence: Hormones, performance, and response to environmental change. *Integrative and Comparative Biology*, **49**, 365-379.

Kitay, J. I. 1963. Pituitary-adrenal function in rat after gonadectomy and gonadal hormone replacement. *Endocrinology*, **73**, 253-&.

Lund, T. D., Hinds, L. R. & Handa, R. J. 2006. The androgen 5 alpha-dihydrotestosterone and its metabolite 5 alpha-androstan-3 beta,17 beta-diol inhibit the hypothalamo pituitary-adrenal response to stress by acting through estrogen receptor beta-expressing neurons in the hypothalamus. *Journal of Neuroscience*, **26**, 1448-1456.

Moore, M. C. 1991. Application of organizational activation theory to alternative male reproductive strategies - a review. *Hormones and Behavior*, **25**, 154-179.

Moore, M. C. 1988. Testosterone control of territorial behavior - tonic-release implants fully restore seasonal and short-term aggressive responses in free-living castrated lizards. *General and Comparative Endocrinology*, **70**, 450-459.

Moore, M. C. 1987. Castration affects territorial and sexual-behavior of free-living male lizards, sceloporus-jarrovi. *Animal Behaviour*, **35**, 1193-1199.

Moore, M. C. & Thompson, C. W. 1990. *Field endocrinology of reptiles - hormonal-control of alternative male reproductive tactics*.

Moore, M. C. & Marler, C. A. 1988. *Hormones behavior and the environment an evolutionary perspective*.

Moore, M. C. & Marler, C. A. 1987. Effects of testosterone manipulations on nonbreeding season territorial aggression in free-living male lizards, sceloporus-jarrovi. *General and Comparative Endocrinology*, **65**, 225-232.

Nelson, R. J. 2005. *An Introduction to BEHAVIORAL ENDOCRINOLOGY*, Third edn. Sunderland: Sinauer Associates, INC.

Nelson, R. J., Gubernick, D. J. & Blom, J. M. C. 1995. Influence of photoperiod, green food, and water availability on reproduction in male california mice (peromyscus-californicus). *Physiology & Behavior*, **57**, 1175-1180.

Oliveira, R. F., Canário, A. V. M. & Ros, A. F. H. 2008. Hormones and alternative reproductive tactics in vertebrates. In: *Alternative Reproductive Tactics* (Ed. by R. F. Oliveira, M. Taborsky & H. J. Brockmann), pp. 132-173. Cambridge: Camridge University Press.

Phoenix, C. H., Goy, R. W., Gerall, A. A. & Young, W. C. 1959. Organizing action of prenatally administrated tetosterone propionate on the tissues mediating mating behaviour in the female guinea pig. *Endocrinology*, **65**, 369-382.

Piersma, T. & Drent, J. 2003. Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology & Evolution*, **18**, 228-233.

Porlier, M., Charmantier, A., Bourgault, P., Perret, P., Blondel, J. & Garant, D. 2012. Variation in phenotypic plasticity and selection patterns in blue tit breeding time: between- and within-population comparisons. *Journal of Animal Ecology*, **81**, 1041-1051.

Proppe, D. S., Sturdy, C. B. & St Clair, C. C. 2011. Flexibility in Animal Signals Facilitates Adaptation to Rapidly Changing Environments. *Plos One*, **6**.

Rubinow, D. R., Roca, C. A., Schmidt, P. J., Danaceau, M. A., Putnam, K., Cizza, G., Chrousos, G. & Nieman, L. 2005. Testosterone suppression of CRH-stimulated cortisol in men. *Neuropsychopharmacology*, **30**, 1906-1912.

Schoepf, I. & Schradin, C. 2012a. Flexibility in social behaviour and predispositions to change reproductive tactics in African striped mice (Rhabdomys pumilio). *Animal Behaviour*, **84**, 1159-1167.

Schoepf, I. & Schradin, C. 2012b. Better off alone! Reproductive competition and ecological constraints determine sociality in the African striped mouse (Rhabdomys pumilio). *Journal of Animal Ecology*, **81**, 649-656.

Schradin, C. 2008. Differences in prolactin levels between three alternative male reproductive tactics in striped mice (Rhabdomys pumilio). *Proceedings of the Royal Society B-Biological Sciences*, **275**, 1047-1052.

Schradin, C. 2005. When to live alone and when to live in groups : ecological determinants of sociality in the African striped mouse (Rhabdomys pumilio, Sparrman, 1784). *Belgian Journal of Zoology*, **135**, 77-82.

Schradin, C. & Lindholm, A. K. 2011. Relative fitness of alternative male reproductive tactics in a mammal varies between years. *Journal of Animal Ecology*, **80**, 908-917.

Schradin, C. & Yuen, C.-H. 2011. Hormone levels of male African striped mice change as they switch between alternative reproductive tactics. *Hormones and Behavior*, **60**, 676-680.

Schradin, C. & Pillay, N. 2004. The striped mouse (Rhabdomys pumilio) from the succulent karoo, South Africa: A territorial group-living solitary forager with communal breeding and helpers at the nest. *Journal of Comparative Psychology*, **118**.

Schradin, C., Eder, S. & Müller, K. 2012a. Differential investment into testes and sperm production in alternative male reproductive tactics of the African striped mouse (Rhabdomys pumilio). *Hormones and Behavior*, **61**, 686-695.

Schradin, C., König, B. & Pillay, N. 2010. Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515-521.

Schradin, C., Schneider, C. & Yuen, C. H. 2009a. Age at puberty in male African striped mice: the impact of food, population density and the presence of the father. *Functional Ecology*, **23**, 1004-1013.

Schradin, C., Scantlebury, M., Pillay, N. & Koenig, B. 2009b. Testosterone Levels in Dominant Sociable Males Are Lower than in Solitary Roamers: Physiological Differences

between Three Male Reproductive Tactics in a Sociably Flexible Mammal. *American Naturalist*, **173**, 376-388.

Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., Koenig, B. & Pillay, N. 2012b. Social flexibility and social evolution in mammals: a case study of the African striped mouse (Rhabdomys pumilio). *Molecular Ecology*, **21**, 541-553.

Seale, J. V., Wood, S. A., Atkinson, H. C., Harbuz, M. S. & Lightman, S. L. 2004. Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. *Journal of Neuroendocrinology*, **16**, 989-998.

Sharp, P. J. 2005. Photoperiodic regulation of seasonal breeding in birds. In: *Trends in Comparative Endocrinology and Neurobiology* (Ed. by H. Vaudry, E. Roubos, L. Schoofs, G. Fiik & D. Larhammar), pp. 189-199.

Starck, J. M. 1999. Phenotypic flexibility of the avian gizzard: Rapid, reversible and repeated chances of organ size in response to changes in dietary fibre content. *Journal of Experimental Biology*, **202**, 3171-3179.

Steger, R. W., Bartke, A., Goldman, B. D., Soares, M. J. & Talamantes, F. 1983. Effects of short photoperiod on the ability of golden hamster pituitaries to secrete prolactin and gonadotropins in vitro. *Biology of Reproduction*, **29**, 872-878.

Thompson, C. W. & Moore, M. C. 1989. Interactions among hormones, dominance and color in tree lizards. *American Zoologist*, **29**, A98-A98.

Touma, C., Bunck, M., Glasl, L., Nussbaumer, M., Palme, R., Stein, H., Wolferstaetter, M., Zeh, R., Zimbelmann, M., Holsboer, F. & Landgraf, R. 2008. Mice selected for high versus low stress reactivity: A new animal model for affective disorders. *Psychoneuroendocrinology*, **33**, 839-862.

Viau, V. 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. *Journal of Neuroendocrinology*, **14**, 506-513.

Viau, V. & Meaney, M. J. 1996. The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. *Journal of Neuroscience*, **16**, 1866-1876.

Watts, H. E. & Hahn, T. P. 2012. Non-photoperiodic regulation of reproductive physiology in the flexibly breeding pine siskin (Spinus pinus). *General and Comparative Endocrinology*, **178**, 259-264.

Wikelski, M. & Thom, C. 2000. Marine iguanas shrink to survive El Nino - Changes in bone metabolism enable these adult lizards to reversibly alter their length. *Nature*, **403**, 37-38.

Wikelski, M., Hau, M. & Wingfield, J. C. 2000. Seasonality of reproduction in a neotropical rain forest bird. *Ecology*, **81**, 2458-2472.

Wingfield, J. C. 2008. Organization of vertebrate annual cycles: implications for control mechanisms. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 425-441.

Wingfield, J. C. 2005. Flexibility in annual cycles of birds: implications for endocrine control mechanisms. *Journal of Ornithology*, **146**, 291-304.

Wingfield, J. C., Visser, M. E. & Williams, T. D. 2008. Introduction. Integration of ecology and endocrinology in avian reproduction: a new synthesis. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 1581-1588.

Wingfield, J. C., Hegner, R. E., Dufty, A. M. & Ball, G. F. 1990. The challenge hypothesis - theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist*, **136**, 829-846.

Chapter 1

Corticosterone levels correlate with alloparental care in a sex- and age-dependent manner in African striped mice, *Rhabdomys pumilio*

To be submitted

Corticosterone levels correlate with alloparental care in a sex- and age-dependent manner in African striped mice, *Rhabdomys pumilio*

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Abstract

Individuals of cooperatively breeding species have one life history stage characterized by alloparental care. While many studies have contributed to understand the evolutionary reasons why individuals provide care to young that are not their own offspring, the factors influencing and causing alloparental care are less understood. In captive African striped mice, we tested whether age, sex, and hormonal profiles of helpers influenced alloparental care. In captivity we studied 11 family groups, each with two juvenile and two adult helpers. While mothers showed the highest amount of parental care, for the first time we could show that both male and female helpers participated heavily in infant care, and this independently from their age. Fathers showed similar levels of parental care as mothers but not always significantly more than helpers. Although testosterone levels differed significantly between helpers of different age and sex, with adult male helpers showing the highest levels, we did not find any relationships between testosterone and the amount of alloparental care shown (time spent in the nest with the pups and huddling the pups). Corticosterone levels were negatively correlated with alloparental care, but this depended on the sex and the age of helpers.

Keywords: cooperative breeders, testosterone, helpers.

Introduction

In cooperatively breeding species, individuals show one peculiar life history stage during their lifespan, i.e. being non-reproducing helpers in their (often natal) group (Sherman et al. 1995). Helpers are often kin-related to the breeding individuals of their social group, and importantly they provide caregiving (e.g. huddling pups, licking pups, and feeding pups) to young which are not their own offspring - so-called alloparental care (Solomon & French 1997; Koenig & Disckinson 2004). Between and within cooperative breeding species, the amount of alloparental care can differ between philopatric helpers. While many studies investigated these inter-individual differences in alloparental care to ultimately understand why animals show alloparental care (Hamilton 1964; Trivers 1971; Riedman 1982; Balshine-Earn et al. 1998; Koenig & Disckinson 2004), less is known about the proximate mechanisms that cause these differences in caregiving between helpers (Carter & Roberts 1997; Solomon & French 1997; Koenig & Disckinson 2004; Schoech et al. 2004b).

Variation in the expression of alloparental might be due to variation in testosterone and corticosterone levels (Carter & Roberts 1997; Schoech et al. 2004b). In prairie voles, *Microtus ochrogaster*, male and female helpers show low testosterone levels and high corticosterone levels (Carter & Getz 1985; Carter et al. 1986), and a postnatally experimental increase of testosterone reduced alloparental care in males but not in females, whereas a postnatally experimental increase of corticosterone reduced alloparental care in females but not in males (Roberts et al. 1996). These studies indicate that maintaining low testosterone levels might be important for the expression of alloparental care in male helpers. Interestingly, natural variation of testosterone and corticosterone levels in both male and female prairie vole helpers is not associated with differential responses in alloparental care (Roberts et al. 1998). In Mongolian gerbils, *Meriones unguiculatus*, high testosterone levels experienced intrauterine reduce the future expression of alloparental care in males (Clark et al. 1998; Clark & Galef 2000). In male meerkat helpers, *Suricata suricatta*, testosterone levels are negatively correlated to pup feeding (Young et al. 2005). The idea that elevated testosterone levels decrease parental care is generally accepted in birds (Wingfield et al. 1990) and might also be true in alloparental care such as in Aphelocoma jays, *Aphelocoma sp.*, where testosterone

605 levels of male helpers drop when chicks are present in the nest (Vleck & Brown 1999).
606 However, in mammals, effects of testosterone as well as corticosterone on alloparental
607 care seem species and sex specific (Hirschenhauser & Oliveira 2006; Storey et al. 2006).
608 The influence of testosterone levels on alloparental care might be related to age
609 dependent developmental effects. For instance, in pied kingfishers, *Ceryle rudis*, older
610 helpers show higher testosterone levels and provide less alloparental care than younger
611 helpers (Reyer et al. 1986). However, in this species, older helpers are not related to the
612 breeding males (younger helpers are breeding males' offspring) and the high testosterone
613 levels of older helpers could also be a consequence of their aggressive interactions with
614 male breeders (Reyer et al. 1986; Wingfield et al. 1990). In callitrichid primates, older
615 helpers typically show more alloparental care than younger helpers (Price 1992;
616 Yamamoto & Box 1997; Achenbach & Snowdon 1998; Zahed et al. 2010). Although
617 there is evidence that testosterone might play a role in paternal care in common
618 marmosets, *Callithrix jacchus* - fathers show a decrease in testosterone levels when
619 experiencing infant stimuli (Prudom et al. 2008) - to our knowledge, there is no study that
620 investigated the effects of age in relation to testosterone levels and alloparental care in
621 any mammalian species.
622 The amount of alloparental care often differs between the sexes. For instance, in
623 callitrichids, males typically provide more alloparental care than females (Price 1992;
624 Yamamoto & Box 1997; Achenbach & Snowdon 1998; Zahed et al. 2010), although age
625 can interact with the sex effect such as in cotton-top tamarins, *Saguinus oedipus*, where
626 young females show more alloparental care than young males (Price 1992). No sex
627 differences in alloparental care has been found in Goeldi's monkeys, *Callimico goeldii*
628 (Schradin & Anzenberger 2001). In birds females generally help less than males
629 (Cockburn 1998) such as in Florida scrub jays, *Aphelocoma coerulescens* (Hailman et al.
630 1994). In cooperatively breeding fish species, no significant sex difference in alloparental
631 care was reported (Bruintjes & Taborsky 2008; Desjardins et al. 2008). In praire voles,
632 differences in alloparental care were not correlated to sex differences (Roberts et al.
633 1998). In sum, the sex of helpers seems to influence alloparental care in a species
634 dependent manner, but when sex differences in alloparental care exist, the proximate

635 mechanisms underlying this sex-related alloparental care differences are poorly
636 understood.
637 In the present study, we studied variation in alloparental care depending on
638 differences in steroid hormone levels (testosterone and corticosterone), age, and sex in
639 African striped mice (*Rhabdomys pumilio*). This diurnal rodent species shows social
640 flexibility (Schradin et al. 2012) with individuals living either in extended family groups
641 or solitarily, depending on environmental conditions (Schradin et al. 2010a; Schoepf &
642 Schradin 2012a). Groups consist of one territorial dominant male – this male monopolize
643 the reproduction (Schradin et al. 2010b) – up to four breeding females, and philopatric
644 helpers of both sexes (Schradin & Pillay 2004). Philopatric helpers show lower
645 testosterone and higher corticosterone than territorial dominant breeders and testosterone
646 levels increase in male helpers with age (Schradin et al. 2009b; Schradin et al. 2009a).
647 Philopatric female helpers can vary in age between 3 weeks (juveniles) and 3 months
648 (fully adult) (Schradin & Pillay 2004), while male helpers can even be more than 10
649 months old (Schradin et al. 2009b). We first compared steroid hormone levels between
650 male and female helpers of two age classes (juvenile and adult) and breeding males (due
651 to hormonal changes during pregnancy breeding females were not included). We then
652 compared the amount of alloparental care with parental care by both parents and tested
653 whether African striped mouse helpers show sex and age differences in providing
654 alloparental care. We finally tested if inter-individual variations of testosterone and
655 corticosterone levels influenced alloparental care.
656
657 **Materials and methods**
658 *Animals and breeding conditions*
659 The founder pairs of the striped mouse colony set up at the University of Zurich
660 originated from individuals trapped in the Succulent Karoo in South Africa in 2002. Mice
661 were housed under a 11:13h light / dark regime with partly controlled temperature
662 (approx. 23ºC). Family groups were housed in two glass tanks (50x30x30cm) which were
663 connected to one another by a flexible plastic tube. Additionally, one plastic cage
664 (20x13x15cm) was provided with tissue as nesting material. All tanks and cages had 5 cm
665 of wood shavings as bedding. Each group received a seed mixture in the morning (4 g per

/mouse), a fruit or lettuce at midday (1g/mouse), and 2 mealworms / mouse in the afternoon. Water was provided *ad libitum*.

Protocol

Eleven family groups were studied, consisting of one breeding pair and their offspring from three litters. When the siblings of the first two litters were 21 days old, each litter was reduced in number to one female and one male. Thus, when the third litter was born, four helpers of different age and sex were present in each family unit: one male and one female adult helper (59.6 ± 3.3 days old) and one male and one female juvenile helper (29.2 ± 2.5 days old). Each mouse was dyed (Rapido, Pinetown South Africa) for identification with a unique mark on the pelage.

We video-recorded the nests (which were all in the small plastic cage) from the day of the birth of the third litter for 9 consecutive days (D0-D8), each day for 30min. Recordings were done alternatively during the morning between 9:00 am and 12:00am or during the afternoon between 3:00pm and 06:00pm, when striped mice are most active. During recordings, no observer was inside the animal room and to minimize any potential disturbance as a result of the initial camera set-up, the first five minutes of the recording were not analysed. We used EthoLog 2.25© software (Ottoni 2000) to record parental and alloparental care from the last 25min of recording: presence in the nest with pups (second), huddling pups (second), licking pups (second) (Schradin & Pillay 2003). For statistical analyses we used the mean per day of time spent huddling pups, time spent licking pups, time spent in the nest with pups (which also included the time spent huddling pups, and licking pups).

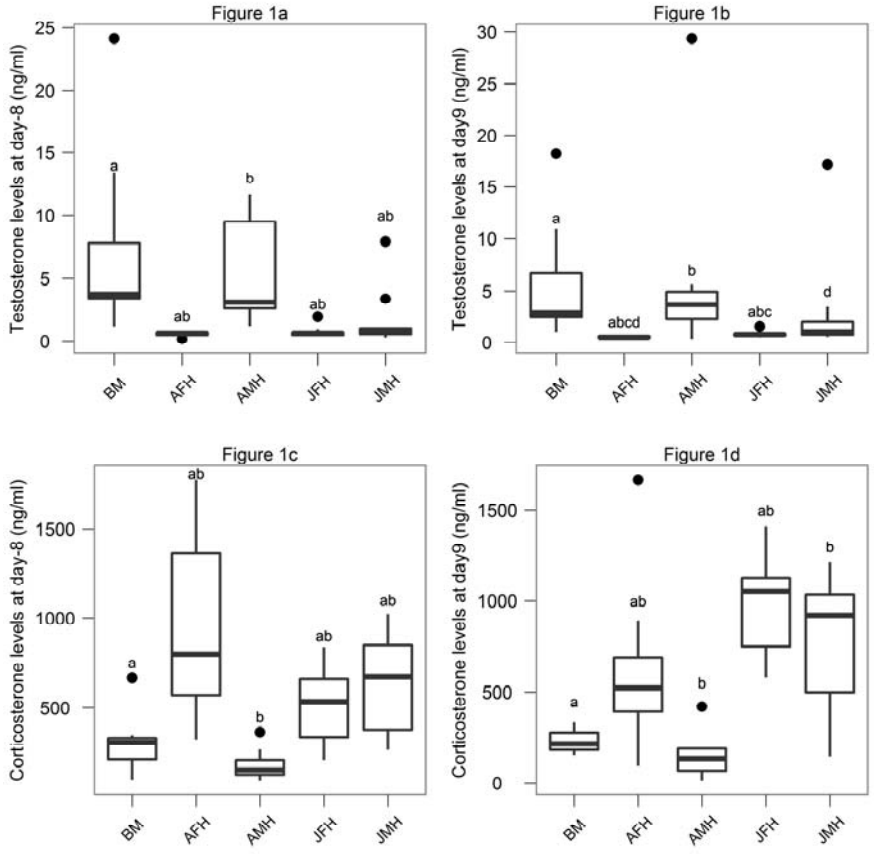
Blood samples

When the offspring of the second litter were 21 days old, a first blood sample was collected from each helper and each breeding male as basal level before birth of the third litter, as the minimum birth interval in striped mice is 23 days (Dewsbury et al. 1984). In practice, this first blood sample was taken 8.2 ± 2.5 (range: 4 – 28 days) days before the birth of the third litter. We took a second blood sample from each helper and each breeding male at the end of the experiment, that is, 9 days after the birth of the third litter

697 (D+9). We named the day when we took the first blood samples: D-8 and the day when
698 we took the second blood samples: D+9.
699 Blood samples were collected in the morning within one hour after the lights went
700 on to control for a possible circadian rhythm of hormone secretions. Mice were
701 anaesthetized with ether and a blood sample of 200µl was collected from the sub-lingual
702 vein (Heimann 2006) within less than three minutes. After one hour, blood samples were
703 centrifuged two successive times for 10 min. The resulting serum was frozen in aliquots
704 of 50 µl for testosterone, and 10 µl for corticosterone assays until used.
705
706 *Hormone assays*
707 We ran three testosterone and three corticosterone assays with commercial kits (IBL
708 Hamburg, Germany), previously validated for striped mice serum (Schradin 2008). Since
709 basal corticosterone levels are very high in striped mice (Schradin 2008), samples for the
710 corticosterone assay were diluted 2: 48 with the zero standards. For three samples of
711 testosterone, the amount of serum aliquots was too small and was thus diluted 1: 1 with
712 the zero standards. The intra-assay coefficients of variation were 1.61 % for testosterone
713 and 3.73 % for corticosterone. The inter-assay coefficients of variation were 1.33 % for
714 testosterone and 8.71 % for corticosterone.
715
716 *Data analyses*
717 During the nine days of alloparental care observation, three adult male helpers were
718 euthanized because of injury caused by the breeding males and females –aggression had
719 started just after the birth of the third litter – reducing the number of days of alloparental
720 care observation to 4 days for these three males. We could also not collect a second blood
721 sample for these three males, reducing the sample size down to eight.
722 Statistical analyses were carried out with R 2.15.0. Results are presented as mean
723 ± SEM and significance was accepted at $\alpha \leq 0.05$. We used non-parametric statistics
724 because of the small sample size (N=11). We performed Friedman Rank Sum Test for
725 hormone level comparisons between the categories of individuals. P-value were adjusted
726 for multiple comparisons following the Benjamini and Hochberg method (Benjamini &

727 Hochberg 1995). For each category of individuals, pairwise comparisons of hormone
728 levels (between D-8 and D+9) were performed with Wilcoxon Rank Sum Test.
729 We ran three generalized linear mixed effect models (GLMMs) with Gaussian
730 error distribution to test whether sex, age, testosterone and corticosterone levels (D+9)
731 and their interactions influenced 1) the time spent in the nest with pups, i.e. response
732 variable of GLMM1; 2) the time spent huddling the pups, i.e. response variable of
733 GLMM2; 3) the time spent licking pups, i.e. response variable of GLMM2. The response
734 variable of each GLMM was square root transformed to achieve linearity of residuals and
735 to remove overdispersion. Family affiliation was added as a random factor for each
736 GLMM. For each GLMM, potential explanatory terms were dropped from the full model
737 sequentially by a backwards stepwise procedure, following (Crawley 2007), omitting
738 non-significant explanatory terms ($p > 0.05$). We accepted a simplified model when it did
739 not differ significantly from its corresponding full model by an ANOVA test ($p > 0.05$).
740
741 **Results**
742 *Testosterone*
743 At D-8, testosterone levels differed significantly between social classes ($N = 11$;
744 Friedman chi-squared = 15.15; $p < 0.001$; Figure 1a). Breeding males and adult male
745 helpers had similar testosterone levels from each other ($p = 0.41$) that were significantly
746 higher than those of juvenile male helpers (both $p < 0.01$), adult female helpers (both $p <$
747 0.001), and juvenile female helpers (both $p < 0.001$). The testosterone levels of adult
748 female helpers did not differ significantly from those of juvenile male helpers ($p = 0.30$)
749 and juvenile female helpers ($p = 0.77$). No other significant differences in testosterone
750 levels between helpers were found (adult female helpers vs. juvenile male helpers: $p =$
751 0.30; adult female helpers vs. juvenile female helpers: $p = 0.77$; juvenile male helpers vs.
752 juvenile female helpers: $p = 0.57$).
753 At D+9, testosterone levels differed significantly between social classes ($N = 8$;
754 Friedman chi-squared = 19.3; $p < 0.001$; Figure 1b). Breeding males and adult male
755 helpers had similar testosterone levels ($p = 0.96$). Breeding males had significantly higher
756 testosterone levels than juvenile female helpers ($p < 0.01$), adult female helpers ($p < 0.01$),
757 and juvenile male helpers, though not significantly ($p < 0.1$). Adult male helpers had

758 significantly higher testosterone levels than juvenile female helpers ($p < 0.05$), adult
759 female helpers ($p < 0.05$), but not than juvenile male helpers ($p = 0.26$). The testosterone
760 levels of adult female helpers were significantly lower than those of juvenile female
761 helpers ($p < 0.5$) and juvenile male helpers ($p < 0.01$). The testosterone levels of juvenile
762 male helpers and juvenile female helpers did not differ significantly ($p = 0.26$).
763 Testosterone levels did not differ significantly between D-8 and D+9 for breeding
764 males ($N = 8$; $W = 43$; $p = 0.28$), adult male helpers ($N = 8$; $W = 37$; $p = 0.65$), adult
765 female helpers ($N = 8$; $W = 33.5$; $p = 0.92$), juvenile male helpers ($N = 8$; $W = 23$; $p =$
766 0.38), and juvenile female helpers ($N = 11$; $W = 21$; $p = 0.27$).
767



768

769 Figure1. Testosterone levels (top) at D-8 (a), at D+9 (b), and corticosterone levels
770 (bottom) at D-8 (c) and at D+9 (d). Breeding males (BM), adult female helpers (AFH),
771 adult male helpers (AMH), juvenile female helpers (JFH), and juvenile male helpers

772 (JMH). The median is indicated by the horizontal bar inside the box, the first and third
773 quartiles by the box itself, outliers are shown as black dots.
774
775 *Corticosterone*
776 At D-8, corticosterone levels differed significantly between social classes (N = 11;
777 Friedman chi-squared = 30.76; $p < 0.001$; Figure 1c). Adult male helpers tended to show
778 lower corticosterone levels than breeding males ($p < 0.1$). Both breeding males and adult
779 male helpers had significantly lower corticosterone levels than juvenile male helpers
780 ($p<0.01$ and $p < 0.001$), adult female helpers (both $p < 0.001$), and juvenile female
781 helpers ($p < 0.05$ and $p < 0.001$). The corticosterone levels of juvenile male helpers did
782 not differ significantly from those of adult female helpers ($p = 0.24$) and juvenile female
783 helpers ($p = 0.27$). Juvenile female helpers tended to show lower corticosterone levels
784 than adult female helpers ($p < 0.1$).
785 At D+9, corticosterone levels differed significantly between social classes (N = 8;
786 Friedman chi-squared = 19.4; $p < 0.001$; Figure 1d). Breeding males and adult male
787 helpers had similar corticosterone levels ($p = 0.13$). Breeding males had significantly
788 lower corticosterone levels than adult female helpers ($p < 0.05$) and juvenile female
789 helpers ($p < 0.001$), and juvenile male helpers, though not significantly ($p < 0.1$). Adult
790 male helpers had significantly lower corticosterone levels than adult female helpers ($p <$
791 0.05), juvenile female helpers ($p < 0.001$), and juvenile male helpers ($p < 0.05$). The
792 corticosterone levels of juvenile male helpers did not differ significantly from those of
793 adult female helpers ($p = 0.42$) and juvenile female helpers ($p = 0.44$). Adult female
794 helpers tended to show lower corticosterone levels than juvenile female helpers ($p < 0.1$).
795 The levels of corticosterone did not differ significantly between D-8 and D+9 for
796 dominant breeding males (N = 8; W = 37; $p = 0.65$), adult male helpers (N = 8; W = 38; p
797 = 0.57), adult female helpers (N = 8; W = 44; $p = 0.23$), and juvenile male helpers (N = 8;
798 W = 24; $p = 0.44$). Corticosterone levels of juvenile female helpers increased
799 significantly from D-8 and D+8 (N = 8; W = 8; $p < 0.05$).
800
801
802

803 *Alloparental and parental care*

804

805 Table 1: adjusted p-values (adj. p-value) for multiple comparisons between all social
806 categories for the time spent in the nest with pups (top), time spent huddling pups (miidle)
807 and time spent licking pups (bottom; mean ± SEM): breeding females (BF), breeding
808 males (BM), adult male helpers (AMH), adult female helpers (AFH), juvenile male
809 helpers (JMH), and juvenile female helper (JFH). Significant differences are marked in
810 bold.

		Adj. p-values				
Category	Time in the nest Time spent huddling pups Time spend licking pups	BF	BM	AMH	AFH	JMH
BF	646.45 ± 92.28 440.02 ± 90.62 35.20 ± 9.06	*	*	*	*	*
BM	522.35 ± 137.49 269.34 ± 80.87 25.15 ± 11.98	0.19 0.12 **<0.05**	*	*	*	*
AMH	154.85 ± 76.03 38.64 ± 26.01 1.80 ± 1.31	**< 0.01** **<0.01** **<0.01**	**< 0.05** >0.05 >0.05	*	*	*
AFH	255.73 ± 78.08 156.44 ± 78.63 8.68 ± 4.07	**< 0.01** **<0.05** **<0.01**	0.46 0.58 0.74	0.19 0.15 0.11	*	*
JMH	189.26 ± 40.10 60.47 ± 22.34 3.68 ± 2.48	**< 0.01** **<0.01** **<0.01**	0.19 0.21 0.12	0.22 0.27 0.99	0.65 0.74 0.12	*
JFH	274.17 ± 130.89 99.10 ± 69.81 2.33 ± 1.24	**0.01** **<0.01** **<0.01**	0.32 0.24 0.19	0.32 0.33 0.50	0.57 0.69 0.27	0.90 0.79 0.51

811

812 Breeding females spent significantly more time in the nest with pups than each category
813 of helpers but not than breeding males (Table 1). Breeding males spent significantly more
814 time in the nest with pups than adult male helpers, but not than any other categories of
815 helpers (Table1). The different categories of helpers did not differ significantly in the
816 time spent in the nest with pups (Table 1).
817 Breeding females huddled pups significantly longer than any types of helpers but
818 not longer than breeding males (Table 1). Breeding males huddled significantly longer
819 than adult male helpers, but not than other categories of helpers (Table 1). The different
820 categories of helpers did not differ significantly in the time they spent huddling pups
821 (Table 1) Breeding females spent significantly more time licking pups than any other
822 social categpry (Table 1). There were no other significant differences (Table 1).
823
824 *Age, sex, testosterone, corticosterone, and alloparental care*
825 In GLMM1, we dropped from the full model the interactions with testosterone and
826 testosterone itself as they did not influence significantly the time spent in the nest with
827 pups. Sex, age, corticosterone levels, the interaction between sex and age, and the
828 interaction between sex and corticosterone tended to influence significantly the time
829 spent in the nest with pups (**sex**: $F_{1,26.0} = 4.16$; $p = 0.05$; **age**: $F_{1,33.5} = 3.57$; $p < 0.1$;
830 **corticosterone**: $F_{1,32.6} = 3.29$; $p < 0.1$; **sex:age**: $F_{1,26.5} = 3.24$; $p < 0.1$; **sex:corticosterone**:
831 $F_{1,25.5} = 3.92$; $p < 0.1$; Figure 2). The interaction between age and corticosterone levels
832 influenced significantly the time spent in the nest with pups (**age:corticosterone**: $F_{1,30.7} =$
833 4.33; $p < 0.05$).
834 In GLMM2, we dropped from the full model the interactions with testosterone
835 and testosterone itself as they did not influence significantly the time spent huddling
836 pups. Sex, age, corticosterone levels influenced significantly and negatively the time
837 spent huddling pups (sex: $F_{1,28.9} = 7.21$; $p < 0.05$; **age**: $F_{1,31.1} = 6.92$; $p < 0.05$;
838 **corticosterone**: $F_{1,33.4} = 5.83$; $p < 0.05$). The interaction between the age of helpers and
839 their sex influenced significantly the time spent huddling pups ($F_{1,29.7} = 4.49$; $p < 0.05$;
840 Figure 3). The interaction between the sex of helpers and their corticosterone levels
841 influenced significantly the time spent huddling pups ($F_{1,28.5} = 7.29$; $p < 0.05$; Figure 3).

The interaction between the age of helpers and the corticosterone levels influenced significantly the time huddling pups ($F_{1,34.0} = 5.25$; $p < 0.05$).

In GLMM3, we dropped from the full model all interactions as they did not influence significantly the time spent licking pups. Sex, age, testosterone and corticosterone levels did not influence significantly the time spent licking pups (**age**: $F_{1,36} = 0.42$; $p = 0.50$; **sex**: $F_{1,36} = 1.9$; $p = 0.20$; **testosterone**: $F_{1,36} = 0.14$; $p = 0.70$; **corticosterone**: $F_{1,36} = 0.51$; $p = 0.50$).

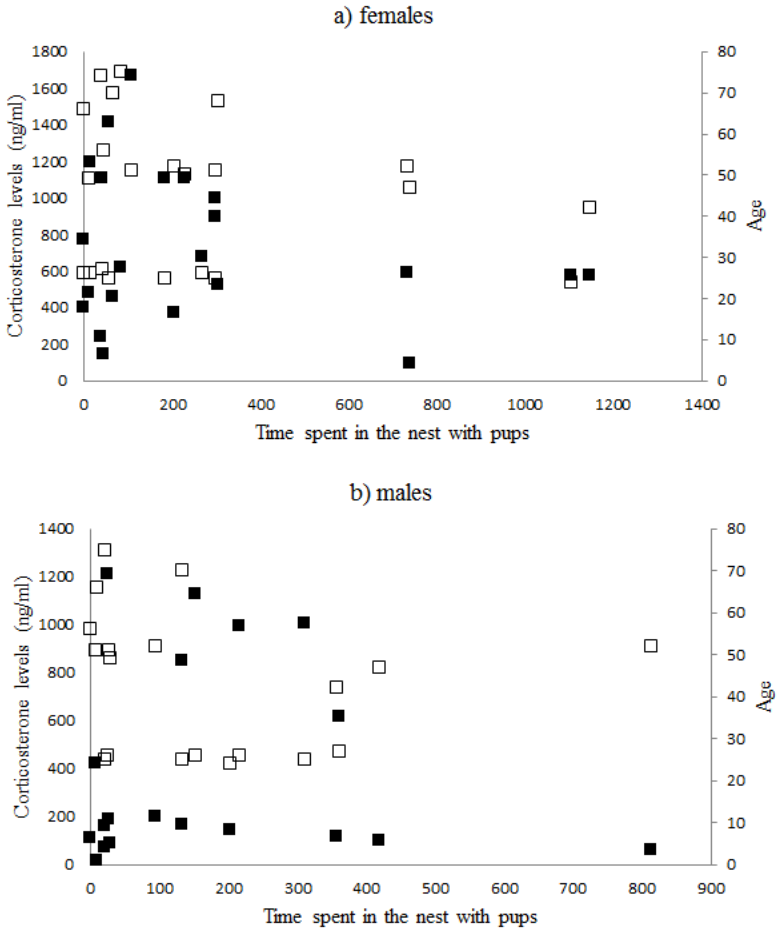


Figure 2. Correlation between corticosterone levels (left Y axis; black squares) at D+9 and the time spent in the nest with pups, and correlation of age (right Y axis; opened squares) at D+9 and the time spent in the nest with pups by helpers: a) in females helpers; b) in male helpers.

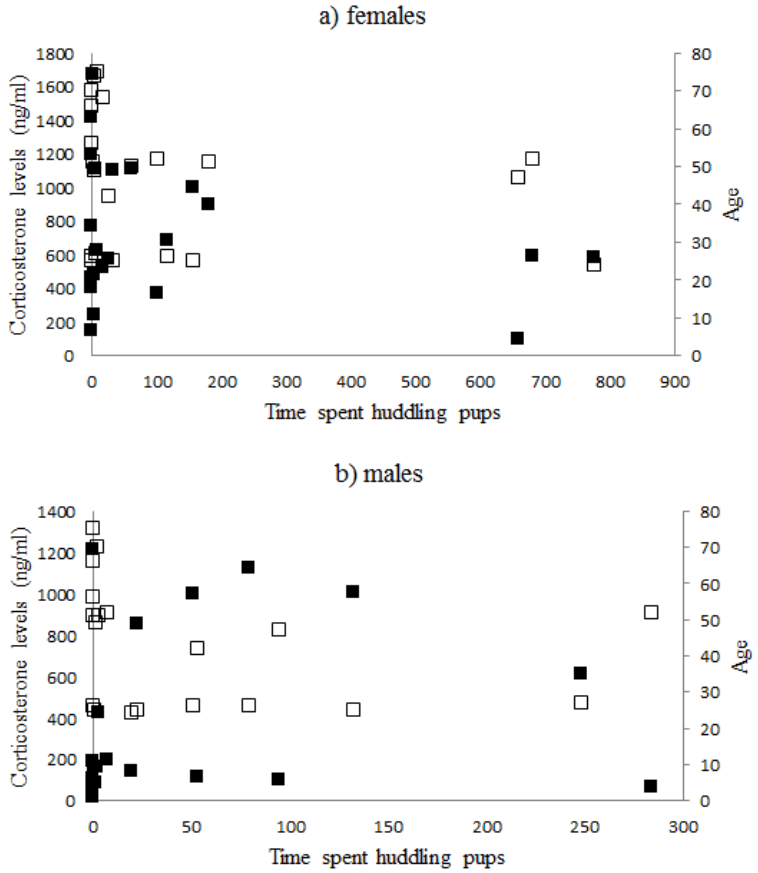


Figure 3. Correlation between corticosterone levels (left Y axis; black squares) at D+9 and the time spent huddling pups, and correlation of age (right Y axis; opened squares) at D+9 and the time spent huddling pups by helpers: a) in females helpers; b) in male helpers.

Discussion

In the present study, we demonstrated for the first time that both males and females, being juveniles or adults, provide alloparental care in African striped mice. This is the first evidence that this communally breeding species (i.e. several females breeding raising their offspring together) (Schubert et al. 2009) show non-reproducing helpers at the nest, as suggested by Schradin and Pillay (2004). Breeding females provided more care to the offspring (time spent in the nest with pups, huddling pups, and licking pups) than any categories of helpers which is in agreement with the general pattern observed in mammal species (Bridges 1990). We also could confirm the important role of paternal care by the

breeding males (Schradin & Pillay 2003), who generally showed more care than helpers. We showed that hormonal profiles differ between helpers of different sex and age, and that sex, age, and corticosterone levels influenced the expression of alloparental care (time spent in the nest with the pups, huddling pups). We did not find any relationships between testosterone and alloparental care.

We observed significant variation in testosterone levels among helpers, with adult male helpers showing higher testosterone levels than other helpers. In pied kingfishers, *Ceryle rudis*, older male helpers also show higher testosterone levels than younger helpers and the high testosterone levels may be due to aggressive interactions with dominant males (Reyer et al. 1986). In our study, we observed aggressive interaction between adult male helpers and breeding males in three out of 11 families. As these three adult male helpers were injured and had to be euthanized before D+9, we do not know their testosterone levels at D+9. The significant increase of testosterone levels in juvenile male helpers from D-8 to D+9 suggests a developmental factor, i.e. the increase of testosterone levels when reaching puberty (Schradin et al. 2009a). Interestingly, the testosterone levels of adult male helpers did not differ significantly from those of breeding males, while juvenile male helpers and female helpers showed lower testosterone levels. In other cooperatively breeding species, dominant breeding males usually have the highest testosterone levels (Wingfield et al. 1990; Oliveira et al. 2005). In a free-ranging striped mouse population, territorial dominant breeding males had higher testosterone levels than adult philopatric males (Schradin et al. 2009b; Schradin & Yuen 2011). The daily food provisioning under our captive conditions may allow male helpers to increase and maintain high testosterone levels, an effect that was found in Florida scrub-jays, *Aphelocoma coerulescens* (Schoech et al. 2004a).

We did not find any evidence that testosterone plays a role in the regulation of alloparental care in African striped mice. In other words, natural variation of testosterone levels in helpers of different sex and age did not influence alloparental care. In a previous study, Raynaud and Schradin (chapter 2) demonstrated that an experimental increase of testosterone levels in male philopatric helpers did not decrease the amount of alloparental care. Our present results are in agreement with this experimental study, overall suggesting no significant effect of testosterone on alloparental care in male African

striped mice. In other rodent species, male philopatric helpers differing in testosterone levels differ in the time they spend huddling pups (Carter & Getz 1985; Carter et al. 1986; Roberts et al. 1996; Clark et al. 1998; Clark & Galef 2000) indicating a role for testosterone in the expression of alloparental care in these studied species. Our result thus supports the idea that testosterone influences on alloparental care are species specific in mammals (Hirschenhauser & Oliveira 2006; Storey et al. 2006). In Puerto Rican frogs, *Eleutherodactylus coqui* (Townsend et al. 1991), and in chestnut-collared longspurs, *Calcarius ornatus* (Lynn et al. 2002), male caregiving was not found to be influenced by testosterone. Alternatively, whether or not testosterone influences male caregiving might also depend on the peculiar environmental or social context (Lynn 2008; Gleason et al. 2009). For instance, in African Striped mice, helpers that dispersed showed an increase of testosterone levels after they dispersed (Schoepf and Schradin, submitted) and at the same time were more aggressive towards pups than individuals that remained helpers in their natal group (Schoepf & Schradin 2012b).

All female and juvenile male helpers had higher corticosterone levels than breeding males. This result suggests signs of reproductive suppression in juvenile males (Schradin et al. 2009a). By contrast, female helper striped mice are not reproductively suppressed (Schradin, under preparation; Schradin et al. 2010b) and their corticosterone levels are not higher than those of breeding females (Schradin 2008). The corticosterone levels of helpers correlated significantly with the time spent huddling pups, and, though not significantly, with the time spent in the nest, but not with the time spent licking pups. The negative relationship between corticosterone levels and the time spent huddling pups was only found for females, but not for males. In prairie voles, a postnatally experimental increase of corticosterone reduced the time spent in contact with pups in female but not in male helpers (Roberts et al. 1996). The influence of corticosterone levels on alloparental care (both time spent in the nest with pups and huddling pups) also depended on the age of helpers and this interaction depended on whether the helper is a male or a female. Thus, in female helpers both age and corticosterone levels had a negative influence on the time spent huddling pups, i.e. younger female helpers with lower corticosterone levels showed more alloparental care. In males helpers, corticosterone levels and age had opposite influences on both the time spent in the nest with pups and huddling pups, that is,

younger male helpers with higher corticosterone showed more alloparental care. Our data suggest that different corticosterone-related mechanisms between sexes might be at play in the regulation of alloparental care. Corticosterone secretion can be part of the regulation of parental care (Angelier & Chastel 2009; Bokony et al. 2009), such as in black-legged kittiwakes, *Rissa tridactyla* (Angelier et al. 2009). For instance the stress response (corticosterone secretion) associated with changes in environmental conditions allow parents to adjust efforts in parental care in terms of their energetics (Angelier et al. 2009). In African striped mice, the influence of corticosterone levels on alloparental care might also be associated with variation in energetic expenditures associated to changes in environmental conditions.

Conclusion

We found alloparental care in juvenile and adult male and female striped mice that stayed as non-breeders in their family group, as suggested from field observations by Schradin and Pillay (2004). Both natural variation in testosterone levels (this study) and an experimental increase of testosterone levels in captive helpers (chapter 2) did not influence alloparental care. These results suggest that testosterone plays no role in the regulation of alloparental care under the standardized captive environment. Our results found indication for a role of corticosterone in alloparental care in helpers in an age- and sex-dependent manner, though this was only significant for huddling pups. It remains to test experimentally whether corticosterone really negatively influences alloparental care in female helpers but not in males and whether the effects of corticosterone manipulation on the time spent huddling pups change with the age of helpers.

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References

Achenbach, G. G. & Snowdon, C. T. 1998. Response to sibling birth in juvenile cotton-top tamarins (Saguinus oedipus). *Behaviour*, **135**, 845-862.

Angelier, F. & Chastel, O. 2009. Stress, prolactin and parental investment in birds: A review. *General and Comparative Endocrinology*, **163**, 142-148.

Angelier, F., Clement-Chastel, C., Welcker, J., Gabrielsen, G. W. & Chastel, O. 2009. How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. *Functional Ecology*, **23**, 784-793.

Balshine-Earn, S., Neat, F. C., Reid, H. & Taborsky, M. 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology*, **9**, 432-438.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological*, **57**, 289-300.

Bokony, V., Lendvai, A. Z., Liker, A., Angelier, F., Wingfield, J. C. & Chastel, O. 2009. Stress Response and the Value of Reproduction: Are Birds Prudent Parents? *American Naturalist*, **173**, 589-598.

Bruintjes, R. & Taborsky, M. 2008. Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex. *Animal Behaviour*, **75**, 1843-1850.

Carter, C. S. & Roberts, R. L. 1997. The psychobiological basis of cooperative breeding in rodents. In: *Cooperative Breeding in Mammals* (Ed. by N.G. Salomon & J. A. French). Cambridge: Cambridge University Press.

Carter, C. S. & Getz, L. L. 1985. *Social and hormonal determinants of reproductive patterns in the prairie vole*.

Carter, C. S., Getz, L. L. & Cohenparsons, M. 1986. Relationships between social-organization and behavioral endocrinology in a monogamous mammal. *Advances in the Study of Behavior*, **16**, 109-145.

Clark, M. M. & Galef, B. G. 2000. Why some male Mongolian gerbils may help at the nest: testosterone, asexuality and alloparenting. *Animal Behaviour*, **59**, 801-806.

Clark, M. M., Vonk, J. M. & Galef, B. G. 1998. Intrauterine position, parenting, and nest-site attachment in male Mongolian gerbils. *Developmental Psychobiology*, **32**, 177-181.

Cockburn, A. 1998. Evolution of helping behavior in cooperatively breeding birds. *Annual Review of Ecology and Systematics*, **29**, 141-177.

Crawley, M. 2007. *Statistics An Introduction using R*. West Sussex: Wiley.

Desjardins, J. K., Stiver, K. A., Fitzpatrick, J. L., Milligan, N., Van Der Kraak, G. J. & Balshine, S. 2008. Sex and status in a cooperative breeding fish: behavior and androgens. *Behavioral Ecology and Sociobiology*, **62**, 785-794.

Dewsbury, D. A., Ferguson, B. & Webster, D. G. 1984. Aspects of reproduction, ovulation, and the estrous-cycle in african 4-striped grass mice (rhabdomys-pumilio). *Mammalia*, **48**, 417-424.

Gleason, E. D., Fuxjager, M. J., Oyegbile, T. O. & Marler, C. A. 2009. Testosterone release and social context: When it occurs and why. *Frontiers in Neuroendocrinology*, **30**, 460-469.

Hailman, J. P., McGowan, K. J. & Woolfenden, G. E. 1994. Role of helpers in the sentinel behavior of the florida scrub jay (aphelocoma c coerulescens). *Ethology*, **97**, 119-140.

Hamilton, W. D. 1964. Genetical evolution of social behaviour 2. *Journal of Theoretical Biology*, **7**, 1-52.

Heimann, M. 2006. Development and validation of the method of sublingual blood sampling in mice and other small rodents, University of Zurich.

Hirschenhauser, K. & Oliveira, R. F. 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Animal Behaviour*, **71**, 265-277.

Koenig, W. & Disckinson, J. 2004. Evolutionary origins. In: *Ecology and Evolution of Cooperative Breeding in Birds* (Ed. by W. Koenig & J. Disckinson). Cambridge: Cambridge University Press.

Lynn, S. E. 2008. Behavioral insensitivity to testosterone: Why and how does testosterone alter paternal and aggressive behavior in some avian species but not others? *General and Comparative Endocrinology*, **157**, 233-240.

Lynn, S. E., Hayward, L. S., Benowitz-Fredericks, Z. M. & Wingfield, J. C. 2002. Behavioural insensitivity to supplementary testosterone during the parental phase in the chestnut-collared longspur, - Calcarius ornatus. *Animal Behaviour*, **63**, 795-803.

Oliveira, R. F., Ros, A. F. H. & Goncalves, D. M. 2005. Intra-sexual variation in male reproduction in teleost fish: a comparative approach. *Hormones and Behavior*, **48**, 430-439.

Ottoni, E. B. 2000. EthoLog 2.2: A tool for the transcription and timing of behavior observation sessions. *Behavior Research Methods Instruments & Computers*, **32**, 446-449.

Price, E. C. 1992. Contributions to infant care in captive cotton-top tamarins (saguinus-oedipus) - the influence of age, sex, and reproductive status. *International Journal of Primatology*, **13**, 125-141.

Prudom, S. L., Broz, C. A., Schultz-Darken, N., Ferris, C. T., Snowdon, C. & Ziegler, T. E. 2008. Exposure to infant scent lowers serum testosterone in father common marmosets (Callithrix jacchus). *Biology Letters*, **4**, 603-605.

Reyer, H. U., Dittami, J. P. & Hall, M. R. 1986. Avian helpers at the nest - are they psychologically castrated. *Ethology*, **71**, 216-228.

Riedman, M. L. 1982. The evolution of alloparental care and adoption in mammals and birds. *Quarterly Review of Biology*, **57**, 405-435.

Roberts, R. L., Miller, A. K., Taymans, S. E. & Carter, C. S. 1998. Role of social and endocrine factors in alloparental behavior of prairie voles (Microtus ochrogaster). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **76**, 1862-1868.

Roberts, R. L., Zullo, A., Gustafson, E. A. & Carter, C. S. 1996. Perinatal steroid treatments alter alloparental and affiliative behavior in prairie voles. *Hormones and Behavior*, **30**, 576-582.

Schoech, S. J., Bowman, R. & Reynolds, S. J. 2004a. Food supplementation and possible mechanisms underlying early breeding in the Florida Scrub-Jay (Aphelocoma coerulescens). *Hormones and Behavior*, **46**, 565-573.

Schoech, S. J., Reynolds, S. J. & Boughton, R. K. 2004b. Endocrinology. In: *Ecology and Evolution of Cooperative Breeding in Birds* (Ed. by W. Koenig & J. Dickinson). Cambridge: Cambridge University Press.

Schoepf, I. & Schradin, C. 2012a. Better off alone! Reproductive competition and ecological constraints determine sociality in the African striped mouse (Rhabdomys pumilio). *Journal of Animal Ecology*, **81**, 649-656.

Schoepf, I. & Schradin, C. 2012b. Flexibility in social behaviour and predispositions to change reproductive tactics in African striped mice (Rhabdomys pumilio). *Animal Behaviour*, **84**, 1159-1167.

Schradin, C. 2008. Seasonal changes in testosterone and corticosterone levels in four social classes of a desert dwelling sociable rodent. *Hormones and Behavior*, **53**, 573-579.

Schradin, C. & Yuen, C.-H. 2011. Hormone levels of male African striped mice change as they switch between alternative reproductive tactics. *Hormones and Behavior*, **60**, 676-680.

Schradin, C. & Pillay, N. 2004. The striped mouse (Rhabdomys pumilio) from the succulent karoo, South Africa: A territorial group-living solitary forager with communal breeding and helpers at the nest. *Journal of Comparative Psychology*, **118**, 37-47.

Schradin, C. & Pillay, N. 2003. Paternal care in the social and diurnal striped mouse (Rhabdomys pumilio): Laboratory and field evidence. *Journal of Comparative Psychology*, **117**, 317-324.

Schradin, C. & Anzenberger, G. 2001. Infant carrying in family groups of Goeldi's monkeys (Callimico goeldii). *American Journal of Primatology*, **53**, 57-67.

Schradin, C., König, B. & Pillay, N. 2010a. Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515-521.

Schradin, C., Schneider, C. & Lindholm, A. K. 2010b. The nasty neighbour in the striped mouse (Rhabdomys pumilio) steals paternity and elicits aggression. *Frontiers in Zoology*, **7**, 19.

Schradin, C., Schneider, C. & Yuen, C. H. 2009a. Age at puberty in male African striped mice: the impact of food, population density and the presence of the father. *Functional Ecology*, **23**, 1004-1013.

Schradin, C., Scantlebury, M., Pillay, N. & Koenig, B. 2009b. Testosterone Levels in Dominant Sociable Males Are Lower than in Solitary Roamers: Physiological Differences between Three Male Reproductive Tactics in a Sociably Flexible Mammal. *American Naturalist*, **173**, 376-388.

1082 **Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., König, B. &**
1083 **Pillay, N.** 2012. Social flexibility and social evolution in mammals: a case study of the
1084 African striped mouse (Rhabdomys pumilio). *Molecular Ecology*, **21**, 541-553.
1085 **Schubert, M., Pillay, N. & Schradin, C.** 2009. Parental and alloparental care in a
1086 polygynous mammal. *Journal of Mammalogy*, **90**, 724-731.
1087 **Sherman, P. W., Lacey, E. A., Reeve, H. K. & Keller, L.** 1995. The eusociality
1088 continuum. *Behavioral Ecology*, **6**, 102-108.
1089 **Solomon, N. G. & French, J. A.** 1997. The study of mammalian cooperative breeding.
1090 In: *Cooperative Breeding In Mammals.* (Ed. by N. G. Solomon & J. A. French), pp. 1-10.
1091 New York: Cambridge University Press.
1092 **Storey, A. E., Delahunty, K. M., McKay, D. W., Walsh, C. J. & Wilhelm, S. I.** 2006.
1093 Social and hormonal bases of individual differences in the parental behaviour of birds and
1094 mammals. *Canadian Journal of Experimental Psychology-Revue Canadienne De*
1095 *Psychologie Experimentale*, **60**, 237-245.
1096 **Townsend, D. S., Palmer, B. & Guillette, L. J.** 1991. The lack of influence of
1097 exogenous testosterone on male parental behavior in a neotropical frog (eleutherodactylus)
1098 - a field experiment. *Hormones and Behavior*, **25**, 313-322.
1099 **Trivers, R. L.** 1971. Evolution of reciprocal altruism. *Quarterly Review of Biology*, **46**,
1100 35-&.
1101 **Vleck, C. M. & Brown, J. L.** 1999. Testosterone and social and reproductive behaviour
1102 in Aphelocoma jays. *Animal Behaviour*, **58**, 943-951.
1103 **Wingfield, J. C., Hegner, R. E., Dufty, A. M. & Ball, G. F.** 1990. The challenge
1104 hypothesis - theoretical implications for patterns of testosterone secretion, mating systems,
1105 and breeding strategies. *American Naturalist*, **136**, 829-846.
1106 **Yamamoto, M. E. & Box, H. O.** 1997. The role of non-reproductive helpers in infant
1107 care in captive Callithrix jacchus. *Ethology*, **103**, 760-771.
1108 **Young, A. J., Carlson, A. A. & Clutton-Brock, T.** 2005. Trade-offs between
1109 extraterritorial prospecting and helping in a cooperative mammal. *Animal Behaviour*, **70**,
1110 829-837.

Zahed, S. R., Kurian, A. V. & Snowdon, C. T. 2010. Social Dynamics and Individual Plasticity of Infant Care Behavior in Cooperatively Breeding Cotton-Top Tamarins. *American Journal of Primatology*, **72**, 296-306.

1114

Chapter 2

Experimental increase of testosterone increases boldness and decreases anxiety in male African striped mouse helpers

Physiology and Behavior (submitted)

Experimental increase of testosterone increases boldness and decreases anxiety in male African striped mouse helpers

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Abstract

Males of many species can adjust their behaviors to environmental conditions by changing reproductive tactics. Testosterone surges in adult breeding males typically inhibit the expression of paternal care while facilitating the expression of aggression during environmental changes. Similarly, in non-breeding philopatric males of cooperatively breeding species, up-regulation of testosterone may inhibit alloparental care while facilitating dispersal, i.e. males might become bolder and more explorative. We tested this hypothesis in philopatric male African striped mice, *Rhabdomys pumilio*. Striped mouse males can either remain in their natal group providing alloparental care or they can disperse seeking mating opportunities. Compared to philopatric males, dispersed males typically show higher testosterone levels and lower corticosterone levels, and more aggression towards pups and same sex conspecifics. We experimentally increased the testosterone levels of philopatric males kept in their family group when pups were present. Testosterone-treated males did not differ significantly from control males in alloparental care and in aggression toward same-sex conspecifics. Compared to control males, testosterone treated males were bolder, more active, less anxious; they also showed lower corticosterone levels. Philopatric males were sensitive to our testosterone treatment for dispersal- and anxiety-like behavior but insensitive for social behaviors. Our results suggest a role of testosterone in dispersal.

Keywords: social flexibility, strategy, cooperative breeding, exploration, shyness, dispersal, solitary-living, roamer.

Introduction

The ability of an individual to change its reproductive tactic as a response to changes in the social and non-social environment can provide numerous advantages (Lott 1991). In several species, males unable to compete with larger males with greater competitive abilities adopt alternative reproductive tactics (ARTs) (Gross 1996; Taborsky 1997; Oliveira et al. 2005). ARTs are discontinuous behavioral and other traits selected to maximize fitness in two or more alternative ways (Oliveira et al. 2008). If the environment and individual body conditions do not favor dispersal, males of social species can remain in their natal group as philopatric helpers providing care toward the breeder's offspring (i.e. alloparental care), instead of dispersing and seeking mating opportunities (Schradin et al. 2012). Males are predicted to disperse and follow reproductive tactics with higher fitness when certain conditions change, e.g. their environment or their competitive ability (Schradin & Lindholm 2011). For instance, a decrease in population density enhances dispersal in philopatric African striped mice, *Rhabdomys pumilio* (Schoepf & Schradin 2012). Dispersal implies essential behavioral shifts, i.e. decreased alloparental care and increased behaviors facilitating dispersal. However, less is known about the proximate mechanisms mediating these adaptive behavioral changes which are thought to rely on hormone-based mechanisms (Moore 1991; Moore et al. 1998).

Androgen levels, for instance testosterone, rise during puberty and correlate with dispersal in many species (Nelson 2005). In Belding's ground squirrels, *Spermophilus beldingi*, early testosterone exposure caused inter-individual dispersal differences (Holekamp et al. 1984; Nunes et al. 1999). However, no study has demonstrated that testosterone surges cause dispersal at later life history stages (e.g. sexual maturity). Yet, dispersal is a risky undertaking (Metzgar 1967; Wolf 1994; Solomon 2003) and the anxiolytic effect of testosterone (Aikey et al. 2002) may facilitate dispersal. Similarly, Holekamp (Holekamp et al. 1984) suggested that testosterone may cause dispersal through the facilitation of exploratory behavior. In African striped mice, Raynaud et al (Raynaud et al. 2012) showed that testosterone-treated juvenile males expanded their home ranges and showed decreased corticosterone levels. As the final decision to disperse may rely on ecological factors (population density and reproductive competition)

(Schradin et al. 2010b; Schradin & Lindholm 2011; Schoepf & Schradin 2012), an increase of testosterone levels may facilitate dispersal through both the inhibition of anxiety-like behavior and a decrease in glucocorticoid levels.

Changes in testosterone levels may also be a mechanism mediating the expression of alloparental care in cooperatively breeding species (Schoech et al. 2004). Elevated testosterone levels typically decrease paternal care in birds (Wingfield et al. 1990), although this testosterone action seems more species specific in mammalian fathers (Hirschenhauser & Oliveira 2006; Storey et al. 2006). Prolactin levels correlate positively with alloparental care in different species, such as the Florida scrub-jay, *Aphelocoma c. coerulescens* (Schoech et al. 1996). However, in other species, helpers show both low prolactin and low testosterone levels, such as the African striped mouse (Schradin & Yuen 2011). This suggests that low testosterone levels may facilitate alloparental care instead of high prolactin levels. In prairie voles, *Microtus ochrogaster*, Roberts et al (Roberts et al. 1996) demonstrated that testosterone administrated postnatally decreased the alloparental responsiveness of males. Thus, while up-regulation of the hypothalamus pituitary gonadal axis (HPG) resulting in increased testosterone levels may facilitate dispersal-like behavior, high testosterone levels may result in a decrease of alloparental care. Consistent with this hypothesis, Young et al (Young et al. 2005) demonstrated, in meerkats (*Suricata suricata*), that male helpers showed high testosterone levels and decreased pup feeding rates after prospecting for dispersing opportunities. However, experimental demonstration of the role of testosterone in mediating both behaviors facilitating dispersal and alloparental care is lacking.

In the present study, we tested the role of testosterone in social behavior (alloparental care, affiliative and aggressive behaviors) and in behavior, which may facilitate dispersal, in the African striped mouse. Males striped mice can follow one of three ARTs, accompanied by changes in hormonal profile and parental care: 1) philopatric group-living males showing the lowest testosterone levels, highest corticosterone levels and alloparental care; 2) solitary-living roamers showing the highest testosterone levels, low corticosterone levels and no parental care; and 3) social dominant group-living territorial breeders showing intermediate testosterone levels, low corticosterone levels and high parental care (Schradin & Pillay 2003; Schradin et al.

2009b). Juvenile males can disperse or alternatively they can remain in their natal group (Schradin & Pillay 2005). Philopatric group-living males have to disperse from their natal group to become either solitary-living roamers or dominant group-living territorial breeders (Schradin et al. 2009b; Schradin & Lindholm 2011). Testosterone levels increased when philopatric group-living males changed into solitary-living roamers (Schradin & Yuen 2011). Furthermore, Schoepf and Schradin (Schoepf & Schradin accepted) demonstrated that philopatric males became more aggressive toward same-sexed conspecifics and pups after they dispersed. These studies suggest that an increase of testosterone levels may inhibit the expression of alloparental care and enhance the expression of aggressive behavior. As dispersing males showed increased testosterone and decreased corticosterone levels (Schoepf and Schradin, in preparation), we hypothesize that an increase of testosterone levels may cause philopatric group-living males to be bolder (i.e. more prone to undertake risky behavior (Wilson et al. 1994)), more explorative (i.e. more prone to approach a novel object (Powell et al. 2004)), and less anxious (i.e. more prone to spend time in the anxiogenic open arms than in the safer closed arms of an elevated plus maze (Pellow et al. 1985)). We referred these different behaviors as "dispersal-like behavior". We tested these predictions under standardized conditions in captivity by experimentally increasing testosterone levels of philopatric group-living males.

Materials and methods

Animals and breeding conditions

The founder pairs of the striped mouse colony housed at the University of Zurich originated from individuals trapped in the Succulent Karoo in South Africa in 2002. Mice were housed under a 11:13 h light / dark regime with partly controlled temperature (approx. 23ºC). Family groups were housed in two glass tanks (50x30x30cm) which were connected to one another with a flexible plastic tube. Additionally, one plastic cage (20x13x15 cm) was provided with nesting material. All tanks and cages had 5 cm of wood shavings for bedding. Each mouse received a 4 g seed mixture in the morning, a piece of fruit or lettuce at midday (1 g/mouse), and 2 mealworms in the afternoon. Water was provided *ad libitum*.

We used 11 family groups consisting of one dominant group-living territorial breeder, one breeding female and two litters. Philopatry was mimicked by leaving offspring in the family cage. When the offspring of their first litter were 21 days old, the litter was reduced in number to one philopatric group-living female and two philopatric group-living males. Each mouse was dyed for identification with a unique mark on the pelage (Rapido, Pinetown South Africa).

Experimental testosterone manipulation

We started the testosterone treatment on the day of birth of the second litter when philopatric group-living males of the previous litter were 36.0 ± 2.4 days old. Philopatric group-living males were anesthetized with ether and implanted subcutaneously behind the neck using a precision trochar 10 gauge (Innovative Research of America, Sarasota, FL, USA). In each family, one of the two males randomly received one pellet of 3.5 mg testosterone (time-release pellets from Innovative Research of America, Sarasota, FL, USA) referred to as "test male" while his same-litter sibling received an empty pellet (placebo) referred to as "control male".

Blood collection

Blood samples were collected in the morning within one hour after the lights went on to control for a possible circadian rhythm of hormone secretions. Mice were anaesthetized with ether and a blood sample of 200µl was collected from the sub-lingual vein (Heimann 2006) within less than three minutes. After one hour, blood samples were centrifuged two successive times for 10 min. The resulting serum was frozen in aliquots of 50 µl for testosterone, and 10 µl for corticosterone assays until used. Blood samples were collected from each test and control male directly before the implantation (D0), a day after the implantation (D1), nine days after the implantation (D9) and 14 days (D14) after implantation.

Reproductive status and body mass monitoring

Reproductive status (scrotal, i.e. testes fully descended, or non-scrotal) and body mass (in grams) of the test and control males were recorded after each blood sample was taken.

Breeder aggression

In Mongolian gerbils high testosterone levels can trigger the expulsion of philopatric males from their families (Scheibler et al. 2006). In African striped mice, breeding males and females could show aggressive behaviors towards the test males that could influence the expression of alloparental care. We daily recorded for 30 minutes the frequency of aggressive behaviors (i.e. chasing, fighting, and biting) of breeding males and females toward both the test and control males during the whole experiment.

Alloparental care

We performed daily alloparental care observations from D0 until D9. Observations were alternatively performed during the morning (between 9:00 am and 12:00 am) and the next day during the afternoon (between 03:00 pm and 06:00 pm) to cover alloparental care observations during the whole active period of African striped mice. Nests were videotaped for 30 min without any observers inside the animal room. The first five minutes of each video were ignored to minimize any effects of a potential disturbance as a result of the initial camera set-up. Using the software EthoLog 2.25© (Ottoni 2000), we recorded the time spent (in seconds) by each test and control male in the nest, the time spent huddling, and the time spent licking the pups. We also recorded the frequency of carrying pups in the mouth, and retrieving the pups. We considered the total amount of alloparental care provided by each test and control male as the sum of the time spent in the nest, huddling and licking the pups. We finally considered the mean per day (%/day) of huddling, licking the time spent in the nest, and the total amount of alloparental care (i.e. huddling + Licking + time spent in the nest) for statistical analyses.

Behavioral tests

On D10, we performed three successive behavioral tests in the same order for every test and control mouse (see above). Yuen demonstrated that the order of these tests had no significant influence on boldness, activity, exploration and aggression in male African striped mice (unpublished data).

<u>1- Boldness assessment: open field test</u>

The subject was placed in the periphery of a neutral test arena (80x40x60cm made of wood), and observed for five minutes. The time that mice stay close to the wall (thigmotaxis) and activity were used as an indicator of boldness / shyness: low thigmotaxis and high activity indicate an increase of boldness (Sneddon 2003). Thus, the amount of time the mouse spent with at least half a mouse length away from the arena's walls was recorded to assess increased boldness. We also measured the mouse activity. For this, we recorded every 15 seconds whether the mouse was moving (i.e. walking) or immobile in the open area.

2- Exploratory assessment: novel objects test

With the subject inside the same test arena, two novel objects (a fixed and a movable object: rubber tiger and table tennis ball) were placed at the opposite end of the arena. Direct observations were performed during 5 min to record latency to approach (seconds) and sniffing the object (frequency).

3- Aggressiveness assessment: dyadic encounters

Same sex encounter tests were performed in the same test arena. At the beginning, a partition in the middle divided the arena in two compartments. At one side a stimulus animal was placed. Stimulus animals in these tests were males (22-40 days old) housed in sibling groups. In all cases, the focal animals (i.e. test and control males) were bigger than the stimulus animals (38.1 ± 2.6 vs. 22.1 ± 2.1; $N = 16$; $V = 171$; $p < 0.001$), as it is known that dominance is weight related in striped mice (Schradin 2004). The focal animal (i.e. test or control males) was placed on the other side. After a habituation period of 5 min, the partition was removed and the focal animal was observed for 15 min. No damaging fights occurred during any dyadic encounters. The frequency of aggressive behaviors (chasing, fighting, and biting) were recorded. We also recorded the time spent in body contact and the frequency of sniffing and grooming the stimulus animal. This test has been used previously to measure aggression in striped mice from the field (Schradin et al. 2010a) and in captivity (Schradin, unpublished data).

4- Anxiety assessment: elevated plus maze

Test and control males were tested in an elevated plus maze on D12. The elevated plus maze consisted of two anxiogenic open arms and two safe enclosed arms with an open roof, arranged such that the open arms were opposite to each other. The maze was elevated to a height of 100 cm. We videotaped the number of entrance into each arm and the duration of visits inside each arm during 5min. Two indices were used to measure the aversiveness of the open arm: ratio of open arm entries to total arm entries (OER) (Handley & Mithani 1984) and the ratio of time spent in open arms to total time spent in all arms (OTR) (Pellow et al. 1985). The activity of the mice was evaluated using the total arm entries during the trial.

Hormone assays

We performed testosterone and corticosterone assays with commercial kits (IBL Hamburg, Germany), previously validated for striped mice serum (Schradin 2008a). Since corticosterone levels are very high in philopatric group-living males (Schradin et al. 2009b), samples for the corticosterone assay were diluted (2: 48) with the standard 0. For three samples of testosterone, the amount of serum aliquots was too small for hormone assay and was thus diluted (1: 1) with the standard 0. The intra-assay coefficient of variation was 8.98 % for testosterone and 14.84 % for corticosterone.

Data analysis

We stopped the experiments in two families: we observed wounds in test and control males, and these males did not have access to water and food for two consecutive days. These males were removed from their family units and euthanized. This finally reduced the sample size down to nine. Furthermore, in one family, the test male died after the third blood sample (D9). Thus, for this experiment, we did not obtain a last blood sample (D14) and we could not collect behavioral data about boldness, exploration, aggression, and anxiety.

Statistical analyses were carried out with R 2.15.0 (R Development Core Team 2012)(R Development Core Team 2012)(R Development Core Team 2012)(R Development Core Team 2012). Results are presented as mean ± SEM and significance was accepted at $\alpha \leq 0.05$. We used non-parametric statistical analyses due to small

sample sizes. Each pairwise comparison (between test and control males) was performed with paired exact Wilcoxon Signed Rank Tests and Fisher's Exact Test. To test for relationships of boldness and anxiety with activity, we performed Spearman rank correlations.

Results

Serum hormone levels

Before the treatment, test and control males did not significantly differ in testosterone levels (0.97 ± 0.21 ng/ml *vs.* 0.85 ± 0.15 ng/ml; N = 9; V = 29 p = 0.50; Figure 1a). On D1, D9, and D14, test males showed significantly higher testosterone levels than control males (D1: 47.08 ± 3.97ng/ml *vs.* 1.88 ± 0.60 ng/ml; N = 9, V = 45, p = 0.004; D9: 20.32 ± 3.45 ng/ml *vs.* 1.82 ± 0.59 ng/ml; N = 9, V = 45, p = 0.004; D14: 18.64 ± 5.13 ng/ml *vs.* 1.64 ± 0.24 ng/ml; N = 8, V = 36, p = 0.008).

Before the treatment, test and control males tended to differ in corticosterone levels (876.78 ± 118.49 ng/ml *vs.* 510.49 ± 100.69 ng/ml; N = 8; V = 3; p = 0.08; Figure 1b). On D1 and D9, test males showed significantly lower corticosterone levels than control males (D1: 517.97 ± 100.40 ng/ml vs. 1005.13 ± 251.80 ng/ml; N = 9; V = 6; p = 0.05; D9: 331.39 ± 31.82 ng/ml *vs.* 963,23 ± 218.41 ng/ml; N = 9; V = 5; p = 0.04). On D14, test males tended to show lower corticosterone levels than control males (D14: 259.65 ± 38.14 ng/ml *vs.* 971.38 ± 274.44 ng/ml; N = 7, V = 3, p = 0.08).

Reproductive status and body mass

Test males did not differ from control males in body mass before and during the testosterone treatment (D0: 28.92 ± 3.47 g *vs.* 27.94 ± 3.08 g; N = 9, V = 25, p = 0.82; D1: 31.47 ± 3.53 g *vs.* 30.28 ± 3.07 g; N = 9, V = 31, p = 0.34; D9: 34.67 ± 2.34 g *vs.* 34.89 ± 2.76 g; N = 9, V = 20, p = 0.82; D14: 34.03 ± 3.16 g *vs.* 34.60 ± 3.17 g; N = 8, V = 14, p = 0.64). Test males did not differ from control males in reproductive status before and during the testosterone treatment, as most males were scrotal already on D0 (89 % *vs.* 78 %; Fisher's Exact Test: N = 9, p > 0.99), D1 (89 % *vs.* 89 %; Fisher's Exact Test: N = 9, p > 0.99), D9 (100 % *vs.* 89 %; Fisher's Exact Test: N = 9, p > 0.99), and all test and control males were scrotal on D14.

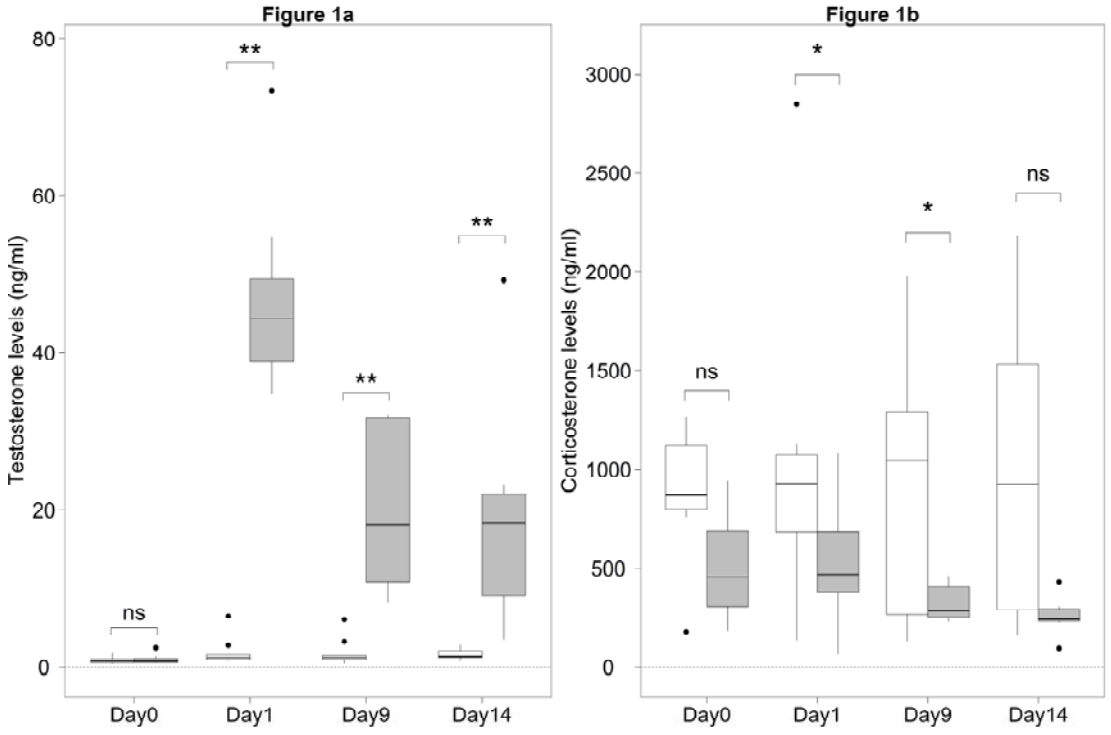

Figure 1. Serum hormone levels before (D0), one day (D1), nine days (D9), and 14 days (D14) after the testosterone treatment in control (white columns) and test males (grey columns); Figure 1a: testosterone; Figure 1b: corticosterone. The median is indicated by the horizontal bar inside the box, the first and third quartiles by the box itself, outliers are shown as black dots. *ns:* non significant; *: $p < 0.05$; **: $p<0.01$.

Breeder aggression

We never observed breeding males and females biting either test or control males. Fighting occurred in one out of nine families and did not differ significantly between test and control males (0.2 ± 0.2 *vs.* 0; N = 9; V = 1; $p > 0.99$). Chasing occurred in three out of nine families and did not differ significantly between test and control males (2.7 ± 2.7 *vs.* 0.8 ± 0.5; N = 9; V = 3; $p > 0.99$).

Alloparental care

The percentage of time that males showed alloparental care did not differ between test males and control males (15.40 ± 5.77 % *vs.* 5.92 ± 2.26 %; N = 9; V = 34; p = 0.20;

Figure 2). Test males tended to huddle the pups longer than control males (12.43 ± 5.23 % *vs.* 3.90 ± 2.30 %; N = 9; V = 38.5; p = 0.07) while there was no difference for the percentage of licking (0.29 ± 0.14 % *vs.* 0.12 ± 0.06 %; N = 9; V = 15; p = 0.40) nor for the percentage of time spent inside the nest (2.69 ± 1.43 % *vs.* 1.89 ± 0.87 %; N = 9; V = 19; p = 0.94).

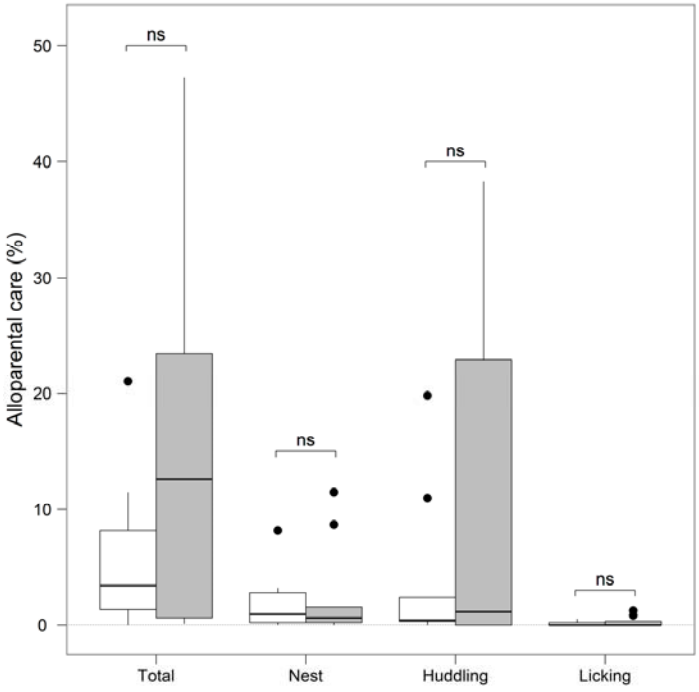

Figure 2. Percentage of time per day that alloparental care was shown by control (white columns) and test males (grey columns): Total = huddling + licking + Nest; Nest = time spent inside the nest with the pups. The median is indicated by the horizontal bar inside the box, the first and third quartiles by the box itself, outliers are shown as black dots. *ns:* non significant.

Boldness, activity (open field test)

Test males tended to spend more time away from the wall than control (52.25 ± 14.77 s *vs.* 26.00 ± 9.61 s; N = 8; V = 31; p = 0.08; Figure 3a). Test males were significantly more active than control males (61.25 ± 10.80 % *vs.* 38.75 ± 8.80 %; N = 9; V = 32.5; p = 0.05; Figure 3b). Both for the test and control males, the time spent away from the wall was significantly positively correlated with activity (test males: $r_s = 0.92$, p = 0.001; control males: $r_s = 0.88$, p = 0.004)

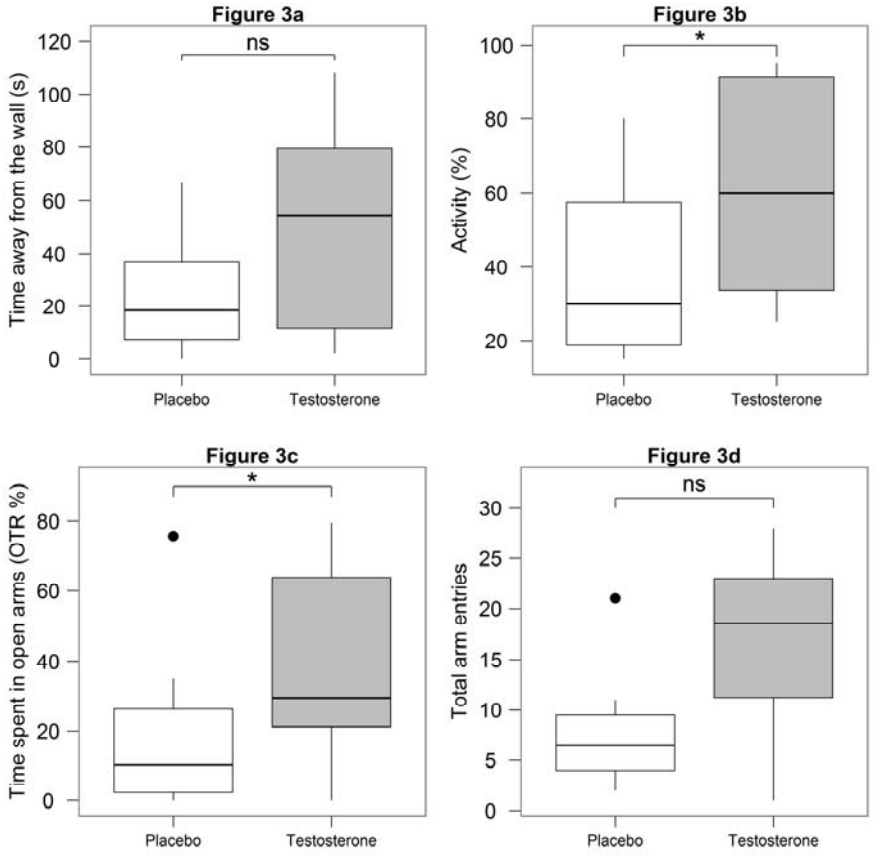

Figure 3. Figure 3a: time (s) away from the wall during the open field test; Figure 3b: activity (%) of the test and control males during the open field test; Figure 3c: time spent in the open arm (OTR; %) during the elevated plus maze test; Figure 3d: total number of arm entries during the elevated plus maze test. The median is indicated by the horizontal bar inside the box, the first and third quartiles by the box itself, outliers are shown as black dots. Placebo (white columns) = control males; Testosterone (grey columns) = test males; *ns:* non significant; *: $p < 0.05$.

Exploration (novel object test)

Neither the latency to approach the fixed nor the mobile object did differ between test and control males (fixed object: 101.13 ± 34.05 s *vs.* 148.13 ± 45.02 s; N = 8; V = 8; p = 0.35; mobile object: 118.88 ± 33.97 s *vs.* 147.75 ± 46.07 s; N = 8; V = 10; p = 0.31). Test males did not sniff the fixed nor the mobile object more often than did control males

(fixed object: 8.50 ± 2.49 *vs.* 4.50 ± 1.89; N = 8; V = 17; p = 0.21; mobile object: 7.38 ± 1.80 *vs.* 4.75 ± 1.57; N = 8; V = 29; p = 0.14).

Aggression (dyadic encounter)

The frequency of aggressive behaviors by test and control males toward the stimulus animal did not differ significantly (2.00 ± 0.82 *vs.* 1.63 ± 0.84; N = 8; V = 10; p = 0.59). The time spent in body contact with the stimulus animal did not differ significantly between test and control males (42.63 ± 34.83 s *vs.* 27.63 ± 20.09 s; N = 8; V = 17; p = 0.94) nor did the frequency of sniffing (8.50 ± 2.35 *vs.* 7.50 ± 2.99; N = 8; V = 17; p = 0.94) and the frequency of grooming the stimulus animal (0.88 ± 0.52 *vs.* 0.63 ± 0.38; N = 8; V = 6; p = 0.86).

Anxiety (elevated plus maze)

Test males spent more time in the open arm (OTR) than control males (39.82 ± 10.34 % *vs.* 19.78 ± 9.06 %; N = 8; V = 28; p = 0.02; Figure 3c). The open entry ratio (OER) did not differ significantly differ between test and control males (36.85 ± 7.58% *vs.* 32.72 ± 7.26 %; N = 8; V = 16; p = 0.29). Test males tended to be more active (total arm entries) than control males (16.88 ± 3.24 *vs.* 8.00 ± 2.12 N = 8; V = 32; p = 0.06; Figure 4d). The OTR was significantly positively correlated with total arm entries for control males (r_s= 0.83, p = 0.01) but not for test males (r_s= 0.19, p = 0.66).

Discussion

Up-regulation of testosterone can decrease the expression of alloparental care (Roberts et al. 1996) and may facilitate dispersal (Schoech et al. 2004). In many cooperative breeding species, juvenile males reaching puberty can either stay in their natal group as helpers showing alloparental care, low androgen and glucocorticoid levels, or they can disperse which is usually positively correlated with an increase of testosterone levels (Schoepf and Schradin, in preparation). However, the extent to which testosterone influences these behavioral changes is still debated (Lynn 2008; Gleason et al. 2009). In the current study, we demonstrated that an experimental increase of testosterone levels in philopatric group-living males increase activity, boldness and decrease anxiety, i.e. traits that may facilitate

dispersal (Holekamp et al. 1984). However, we found no evidence that increased testosterone levels enhance aggressive behavior nor decrease the expression of alloparental care.

The 3.5 mg testosterone pellets increased serum testosterone levels above the maximum physiological levels measured in field samples (15 ng/ml; Schradin unpublished data). This was unexpected as in other rodent species of similar body mass and age, 5 mg testosterone doses resulted in a much lower increase of testosterone levels (in Syrian hamsters: increase from 1ng/ml to 4ng/ml (Romeo et al. 2003); in castrated adult male Rockland-Swiss albino mice: increase to 4.5ng/ml (Barkley & Goldman 1977)). One hypothesis might be species differences in the metabolism of testosterone, i.e. a higher conversion rate of testosterone into its metabolites in Syrian hamsters and wild house mice than in African striped mice. This should be explored in future studies.

In African striped mice, both paternal group-living territorial breeders and non-paternal solitary-living roamers, display high testosterone levels (Schradin & Pillay 2003; Schradin et al. 2009b) but differ in prolactin levels (Schradin 2008b), indicating that prolactin may mediate paternal care (Schradin 2007; Wynne-Edwards & Timonin 2007). However, African striped mouse philopatric males show low prolactin levels which indicates that alloparenting is mediated by different mechanisms (Schradin 2008b). Our results show that test males did not show significantly less alloparenting than control males. Interestingly, test males even tended to huddle pups more often than control males. Our results are surprising because free-ranging dispersing males showing high testosterone levels are more aggressive towards pups than non-dispersing philopatrics males showing low testosterone levels (Schoepf & Schradin accepted) (Schoepf and Schradin, in preparation). As exogenous testosterone alters alloparenting in other mammal species, for instance, in prairie voles (Roberts et al. 1996), our results support the hypothesis that testosterone effects on parental care are species specific in mammals (Storey et al. 2006).

Breeding males and females showed very few aggressive behaviors toward male helpers, and they did not show more aggression towards test than control males. This suggests that the behavior of breeders did not influence the display of alloparental care in our study. Our results also suggest that test males might be insensitive to increased

testosterone levels during the experiment. Similarly, exogenous testosterone did not reduce paternal care in Puerto Rican frogs, *Eleutherodactylus coqui* (Townsend et al. 1991) and in Chestnut-collared longspurs, *Calcarius ornatus* (Lynn et al. 2002). The behavioral insensitivity to testosterone may also explain why test and control males did not differ in aggressive behavior. Thus, our results might be either due to the non-readiness of the brain to respond to testosterone signals (Lynn 2008) or to the adverse effects of testosterone supra-physiological levels produced by 3.5mg testosterone pellets, for example, by downloading androgen receptors (Handa et al. 1994) or estrogen receptor α, which are important in the regulation of social behavior (Cushing et al. 2008).

While we found no significant effect of testosterone on alloparental care and aggression, we observed significant changes in activity, boldness, and anxiety-like behaviors. Test males tended to be bolder and also significantly more active than control males during the open-field tests. While boldness was significantly and positively correlated with activity, test males also spent significantly more time in the open arms of the elevated plus maze regardless of their level of activity (i.e. total arm entries). Thus, increased testosterone levels increase boldness and this indicates a significant anxiolytic effect of testosterone in philopatric male African striped mice. These results contrast with the non-conclusive effects of testosterone on alloparenting and aggression. Activation of androgen receptors in the hypothalamus is necessary to reduce anxiety in mice and rats (Zuloaga et al. 2008; Zuloaga et al. 2011). If the supra-physiological levels of testosterone had down-regulated androgen receptors, reducing brain responsiveness to testosterone, we would expect no significant difference in anxiety-like behavior between test and control males.

Test males showed significantly lower corticosterone levels than control males. Our result indicates that up-regulation of testosterone decreases the basal corticosterone levels of philopatric males. The lower basal corticosterone levels of test males might have decreased the stress response during the open-field test and elevated plus maze test. This suggests that changes in serum testosterone levels mediate the way that African striped mice are coping with stressful situations. In laboratory mice, lower basal corticosterone levels decrease the reactivity of the hypothalamus-pituitary-adrenal (HPA) axis (Touma et al. 2008), and African striped mouse males kept alone showed lower basal

corticosterone levels than males kept with their family (Schradin et al. 2009a). Interestingly, African striped mouse males kept with their family show greater stress response than juvenile males separated from their family and kept individually: these mice showed higher increased corticosterone levels after an elevated plus maze trial and increased anxiety (Mackay and Pillay, in preparation). Overall, our results suggest that testosterone mediates the reactivity of the HPA axis, influencing how philopatric males are coping with stressful situations.

Conclusion

Our results support the role of testosterone in important behavioral and physiological changes associated with male dispersal. Up-regulation of testosterone seems to facilitate dispersal-like behavior either through direct testosterone effects or, perhaps, through the reduction of the reactivity of the HPA axis when philopatric males are coping with dispersing opportunities. These two hypotheses are not mutually exclusive and both involve testosterone actions. We previously demonstrated that an experimental increase of testosterone levels caused juvenile philopatric group-living males to expand their home ranges in African striped mice (Raynaud et al. 2012). However, the decision to disperse relies on other factors; population density and reproductive competition are critical predictors of dispersal in male African striped mice (Schradin et al. 2010b; Schoepf & Schradin 2012). Thus, ecological factors may regulate testosterone signals which facilitate behavioral, physiological, and morphological changes needed to disperse (Raynaud et al. 2012) supporting the role of testosterone in dispersal.

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References

Aikey, J. L., Nyby, J. G., Anmuth, D. M. & James, P. J. 2002. Testosterone rapidly reduces anxiety in male house mice (Mus musculus). *Hormones and Behavior*, **42**, 448-460.

Barkley, M. S. & Goldman, B. D. 1977. Effects of castration and silastic implants of testosterone on intermale aggression in mouse. *Hormones and Behavior*, **9**, 32-48.

Cushing, B. S., Perry, A., Musatov, S., Ogawa, S. & Papademetriou, E. 2008. Estrogen Receptors in the Medial Amygdala Inhibit the Expression of Male Prosocial Behavior. *Journal of Neuroscience*, **28**, 10399-10403.

Gleason, E. D., Fuxjager, M. J., Oyegbile, T. O. & Marler, C. A. 2009. Testosterone release and social context: When it occurs and why. *Frontiers in Neuroendocrinology*, **30**, 460-469.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: Diversity within sexes. *Trends in Ecology & Evolution*, **11**, 92-98.

Handa, R. J., Burgess, L. H., Kerr, J. E. & Okeefe, J. A. 1994. Gonadal-steroid hormone receptors and sex-differences in the hypothalamo-pituitary-adrenal axis. *Hormones and Behavior*, **28**, 464-476.

Handley, S. L. & Mithani, S. 1984. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. *Naunyn-Schmiedebergs Archives of Pharmacology*, **327**, 1-5.

Heimann, M. 2006. Development and validation of the method of sublingual blood sampling in mice and other small rodents. *PhD thesis, University of Zurich*.

Hirschenhauser, K. & Oliveira, R. F. 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Animal Behaviour*, **71**, 265-277.

Holekamp, K. E., Smale, L., Simpson, H. B. & Holekamp, N. A. 1984. Hormonal influences on natal dispersal in free-living belding ground-squirrels (spermophilus-beldingi). *Hormones and Behavior*, **18**, 465-483.

Lott, D. F. 1991. *Intraspecific variation in the social system of wild vertebrates*. New York: Cambridge University Press.

1607 **Lynn, S. E.** 2008. Behavioral insensitivity to testosterone: Why and how does
1608 testosterone alter paternal and aggressive behavior in some avian species but not others?
1609 *General and Comparative Endocrinology*, **157**, 233-240.
1610 **Lynn, S. E., Hayward, L. S., Benowitz-Fredericks, Z. M. & Wingfield, J. C.** 2002.
1611 Behavioural insensitivity to supplementary testosterone during the parental phase in the
1612 chestnut-collared longspur, - Calcarius ornatus. *Animal Behaviour*, **63**, 795-803.
1613 **Metzgar, L. H.** 1967. An experimental comparison of screech owl predation on resident
1614 and transient white-footed mice (peromyscus leucopus). *Journal of Mammalogy*, **48**, 387-
1615 391.
1616 **Moore, M. C.** 1991. Application of organization activation theory to alternative male
1617 reproductive strategies - a review. *Hormones and Behavior*, **25**, 154-179.
1618 **Moore, M. C., Hews, D. K. & Knapp, R.** 1998. Hormonal control and evolution of
1619 alternative male phenotypes: Generalizations of models for sexual differentiation.
1620 *American Zoologist*, **38**, 133-151.
1621 **Nelson, R. J.** 2005. *An Introduction to BEHAVIORAL ENDOCRINOLOGY*, Third edn.
1622 Sunderland: Sinauer Associates, INC.
1623 **Nunes, S., Duniec, T. R., Schweppe, S. A. & Holekamp, K. E.** 1999. Energetic and
1624 endocrine mediation of natal dispersal behavior in Belding's ground squirrels. *Hormones*
1625 *and Behavior*, **35**, 113-124.
1626 **Oliveira, R. F., Canário, A. V. M. & Ros, A. F. H.** 2008. Hormones and alternative
1627 reproductive tactics in vertebrates. In: *Alternative Reproductive Tactics* (Ed. by R. F.
1628 Oliveira, M. Taborsky & H. J. Brockmann), pp. 132-173. Cambridge: Cambridge
1629 University Press.
1630 **Oliveira, R. F., Ros, A. F. H. & Goncalves, D. M.** 2005. Intra-sexual variation in male
1631 reproduction in teleost fish: a comparative approach. *Hormones and Behavior*, **48**, 430-
1632 439.
1633 **Ottoni, E. B.** 2000. EthoLog 2.2: A tool for the transcription and timing of behavior
1634 observation sessions. *Behavior Research Methods Instruments & Computers*, **32**, 446-449.
1635 **Pellow, S., Chopin, P., File, S. E. & Briley, M.** 1985. Validation of open - closed arm
1636 entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of*
1637 *Neuroscience Methods*, **14**, 149-167.

1638 **Powell, S. B., Geyer, M. A., Gallagher, D. & Paulus, M. P.** 2004. The balance between
1639 approach and avoidance behaviors in a novel object exploration paradigm in mice.
1640 *Behavioural Brain Research*, **152**, 341-349.
1641 **R Development Core Team**. 2012. R: A language and environment for statistical
1642 computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-
1643 0, URL http://www.R-project.org/.
1644 **Raynaud, J., Mueller, K. & Schradin, C.** 2012. Experimental increase of testosterone
1645 levels in free-ranging juvenile male African striped mice (Rhabdomys pumilio) induces
1646 physiological, morphological, and behavioral changes. *General and Comparative*
1647 *Endocrinology*, **178**, 108-115.
1648 **Roberts, R. L., Zullo, A., Gustafson, E. A. & Carter, C. S.** 1996. Perinatal steroid
1649 treatments alter alloparental and affiliative behavior in prairie voles. *Hormones and*
1650 *Behavior*, **30**, 576-582.
1651 **Romeo, R. D., Schulz, K. M., Nelson, A. L., Menard, T. A. & Sisk, C. L.** 2003.
1652 Testosterone, puberty, and the pattern of male aggression in Syrian hamsters.
1653 *Developmental Psychobiology*, **43**, 102-108.
1654 **Scheibler, E., Weinandy, R. & Gattermann, R.** 2006. Male expulsion in cooperative
1655 Mongolian gerbils (Meriones unguiculatus). *Physiology & Behavior*, **87**, 27-30.
1656 **Schoech, S. J., Reynolds, S. J. & Boughton, R. K.** 2004. Endocrinology. In: *Ecology*
1657 *and Evolution of Cooperative Breeding in Birds* (Ed. by W. Koenig & J. Dickinson).
1658 Cambridge: Cambridge University Press.
1659 **Schoech, S. J., Mumme, R. L. & Wingfield, J. C.** 1996. Prolactin and helping
1660 behaviour in the cooperatively breeding Florida scrub-jay, Aphelocoma c-coerulescens.
1661 *Animal Behaviour*, **52**, 445-456.
1662 **Schoepf, I. & Schradin, C.** accepted. Flexibility in social behaviour and predispositions
1663 to change reproductive tactics in African striped mice (Rhabdomys pumilio). *Animal*
1664 *Behaviour*.
1665 **Schoepf, I. & Schradin, C.** 2012. Better off alone! Reproductive competition and
1666 ecological constraints determine sociality in the African striped mouse (Rhabdomys
1667 pumilio). *Journal of Animal Ecology*, **81**, 649-656.

1668 **Schradin, C.** 2008a. Seasonal changes in testosterone and corticosterone levels in four
1669 social classes of a desert dwelling sociable rodent. *Hormones and Behavior*, **53**, 573-579.
1670 **Schradin, C.** 2008b. Differences in prolactin levels between three alternative male
1671 reproductive tactics in striped mice (Rhabdomys pumilio). *Proceedings of the Royal*
1672 *Society B-Biological Sciences*, **275**, 1047-1052.
1673 **Schradin, C.** 2007. Comments to K.E. Wynne-Edwards and M.E. Timonin 2007.
1674 Paternal care in rodents: Weakening support of hormonal regulation of the transition to
1675 behavioral fatherhood in rodent animal models of biparental care, Horm & Behav 52 :
1676 114-121. *Hormones and Behavior*, **52**, 557-559.
1677 **Schradin, C.** 2004. Territorial defense in a group living solitary forager: who, where,
1678 against whom? *Behav Ecol Sociobiol*, **55**, 439-446.
1679 **Schradin, C. & Lindholm, A. K.** 2011. Relative fitness of alternative male reproductive
1680 tactics in a mammal varies between years. *Journal of Animal Ecology*, **80**, 908-917.
1681 **Schradin, C. & Yuen, C.-H.** 2011. Hormone levels of male African striped mice change
1682 as they switch between alternative reproductive tactics. *Hormones and Behavior*, **60**, 676-
1683 680.
1684 **Schradin, C. & Pillay, N.** 2005. Intraspecific variation in the spatial and social
1685 organization of the African striped mouse. *Journal of Mammalogy*, **86**, 99-107.
1686 **Schradin, C. & Pillay, N.** 2003. Paternal care in the social and diurnal striped mouse
1687 (Rhabdomys pumilio): Laboratory and field evidence. *Journal of Comparative*
1688 *Psychology*, **117**, 317-324.
1689 **Schradin, C., Schneider, C. & Lindholm, A. K.** 2010a. The nasty neighbour in the
1690 striped mouse (Rhabdomys pumilio) steals paternity and elicits aggression. *Frontiers in*
1691 *Zoology*, **7**, 19.
1692 **Schradin, C., Koenig, B. & Pillay, N.** 2010b. Reproductive competition favours solitary
1693 living while ecological constraints impose group-living in African striped mice. *Journal*
1694 *of Animal Ecology*, **79**, 515-521.
1695 **Schradin, C., Schneider, C. & Yuen, C. H.** 2009a. Age at puberty in male African
1696 striped mice: the impact of food, population density and the presence of the father.
1697 *Functional Ecology*, **23**, 1004-1013.

1698 **Schradin, C., Scantlebury, M., Pillay, N. & Koenig, B.** 2009b. Testosterone Levels in
1699 Dominant Sociable Males Are Lower than in Solitary Roamers: Physiological
1700 Differences between Three Male Reproductive Tactics in a Sociably Flexible Mammal.
1701 *American Naturalist*, **173**, 376-388.
1702 **Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., Koenig, B.**
1703 **& Pillay, N.** 2012. Social flexibility and social evolution in mammals: a case study of the
1704 African striped mouse (Rhabdomys pumilio). *Molecular Ecology*, **21**, 541-553.
1705 **Sneddon, L. U.** 2003. The bold and the shy: individual differences in rainbow trout.
1706 *Journal of Fish Biology*, **62**, 971-975.
1707 **Solomon, N. G.** 2003. A reexamination of factors influencing philopatry in rodents.
1708 *Journal of Mammalogy*, **84**, 1182-1197.
1709 **Storey, A. E., Delahunty, K. M., McKay, D. W., Walsh, C. J. & Wilhelm, S. I.** 2006.
1710 Social and hormonal bases of individual differences in the parental behaviour of birds and
1711 mammals. *Canadian Journal of Experimental Psychology-Revue Canadienne De*
1712 *Psychologie Experimentale*, **60**, 237-245.
1713 **Taborsky, M.** 1997. Bourgeois and parasitic tactics: do we need collective, functional
1714 terms for alternative reproductive behaviours? *Behavioral Ecology and Sociobiology*, **41**,
1715 361-362.
1716 **Touma, C., Bunck, M., Glasl, L., Nussbaumer, M., Palme, R., Stein, H.,**
1717 **Wolferstaetter, M., Zeh, R., Zimbelmann, M., Holsboer, F. & Landgraf, R.** 2008.
1718 Mice selected for high versus low stress reactivity: A new animal model for affective
1719 disorders. *Psychoneuroendocrinology*, **33**, 839-862.
1720 **Townsend, D. S., Palmer, B. & Guillette, L. J.** 1991. The lack of influence of
1721 exogenous testosterone on male parental behavior in a neotropical frog (eleutherodactylus)
1722 - a field experiment. *Hormones and Behavior*, **25**, 313-322.
1723 **Wilson, D. S., Clark, A. B., Coleman, K. & Dearstyne, T.** 1994. Shyness and boldness
1724 in humans and other animals. *Trends in Ecology & Evolution*, **9**, 442-446.
1725 **Wingfield, J. C., Hegner, R. E., Dufty, A. M. & Ball, G. F.** 1990. The challenge
1726 hypothesis - theoretical implications for patterns of testosterone secretion, mating systems,
1727 and breeding strategies. *American Naturalist*, **136**, 829-846.
1728 **Wolf, J. O.** 1994. More on juvenile dispersal in mammals. *Oikos*, **71**, 349-352.

Wynne-Edwards, K. E. & Timonin, M. E. 2007. Paternal care in rodents: Weakening support for hormonal regulation of the transition to behavioral fatherhood in rodent animal models of biparental care. *Hormones and Behavior*, **52**, 114-121.

Young, A. J., Carlson, A. A. & Clutton-Brock, T. 2005. Trade-offs between extraterritorial prospecting and helping in a cooperative mammal. *Animal Behaviour*, **70**, 829-837.

Zuloaga, D. G., Poort, J. E., Jordan, C. L. & Breedlove, S. M. 2011. Male rats with the testicular feminization mutation of the androgen receptor display elevated anxiety-related behavior and corticosterone response to mild stress. *Hormones and Behavior*, **60**, 380-388.

Zuloaga, D. G., Morris, J. A., Jordan, C. L. & Breedlove, S. M. 2008. Mice with the testicular feminization mutation demonstrate a role for androgen receptors in the regulation of anxiety-related behaviors and the hypothalamic-pituitary-adrenal axis. *Hormones and Behavior*, **54**, 758-766.

Chapter 3

Experimental increase of testosterone levels in free-ranging juvenile male African striped mice (*Rhabdomys pumilio*) induces physiological, morphological, and behavioral changes

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Experimental increase of testosterone levels in free-ranging juvenile male African striped mice (*Rhabdomys pumilio*) induces physiological, morphological, and behavioral changes

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Abstract

Testosterone influences sexual differentiation in early development, and activates sexual maturation and sex-related behavior in males during puberty. Testosterone can also influence the expression of male alternative reproductive tactics, by either organizational effects (fixed tactics) or by activational effects (plastic tactics). However, the roles of testosterone in sexual maturation and at the same time the expression of alternative reproductive tactics have been little investigated experimentally, and studies of free-ranging mammals are lacking. We conducted a field experiment in free-ranging juvenile African striped mice (*Rhabdomys pumilio*), a species with alternative reproductive tactics. Juvenile male striped mice reaching puberty can remain in their family as philopatric group-living males with low testosterone levels, or they can disperse and become solitary living roamers with much higher testosterone levels. We tested whether experimentally increased testosterone levels in non-scrotal juvenile males induces puberty and leads to the expression of the roaming tactic. Testosterone-treated males received the hormone for 15 days by silastic implants which were empty in control-

1781 treated males. When compared to control-treated males, testosterone-treated males had
1782 higher testosterone levels, lower corticosterone levels, and became scrotal with
1783 descended testes. Testosterone-treated males also had larger testes, larger epididymides,
1784 and showed indication of spermatogenesis. Testosterone-treated males did not become
1785 solitary-living roamers, but had larger home ranges than control males. We conclude that
1786 testosterone can induce sexual maturation and causes juvenile males to increase their
1787 home ranges, maybe to search for dispersal opportunities.
1788
1789 **Keywords:** sexual maturation; corticosterone; spermatogenesis; dispersal; social
1790 flexibility.

Introduction

Gonadal hormones like testosterone have two types of influences on physiology and behavior, termed "organizational" and "activational" effects (Phoenix et al. 1959). Organizational effects of testosterone typically occur at early developmental stages (i.e. intrauterine life and neonatal period) leading to genital and brain sexual differentiation (Arnold & Breedlove 1985). By contrast, activational effects occur later in life (Arnold & Breedlove 1985). For instance, testosterone surges at puberty, leading to sexual maturation (Nelson 2005) and the expression of sex-related behavior (Beach 1975). Testosterone is important for sexual differentiation, but also for individual differences in morphology, physiology, and behavior within the male sex. The extent of these differences can be determined by both genetic (van Oortmerssen 1971; Sluyter et al. 1995) and environmental factors (Wingfield et al. 1990).

In many animal species, males exhibit alternative reproductive tactics (ARTs) (Gross 1996), that is, discontinuous behavioral and other traits selected to maximize fitness in two or more alternative ways in the context of reproductive competition (Oliveira et al. 2008). The relative plasticity hypothesis suggests that the development of ARTs is regulated by either organizational or activational effects of steroid hormones like testosterone (Moore 1991; Moore et al. 1998). In male species with permanent reproductive tactics, so called fixed ARTs (Moore 1991), a given tactic can be influenced by early exposure to testosterone. In tree lizards, *Urosaurus ornatus*, gonadectomy and testosterone replacement performed at post-hatching determined future reproductive tactics (Hews et al. 1994), while such treatments performed later in life did not affect their tactics (Hews & Moore 1996). These authors concluded that testosterone influences reproductive tactic differentiation by its organisational effect. By contrast, males of other species can change their reproductive tactics later in life, indicating plastic ARTs (Moore 1991). By experimentally changing androgens levels in different species, previous studies demonstrated that these males changed reproductive tactics, i.e. they changed their reproductive behaviors such as territoriality, frequency of copulation, and courtship (Stevenson & Bancroft 1995; Sinervo et al. 2000; Oliveira et al. 2001a; Oliveira et al. 2001b; Peters et al. 2002; de la Cruz et al. 2003; Wikelski et al. 2005; Mills et al. 2009). For example, territorial male marine iguanas, *Amblyrhynchus cristatus* treated with

1822 flutamide (a testosterone and dihydrostestosterone receptor blocker) to block the effects
1823 of testosterone, made them more similar to satellite males (Wikelski et al. 2005). These
1824 males decreased their ability to defend a territory, which ultimately decreased their fitness
1825 (Wikelski et al. 2005). This indicates that the development of reproductive tactics was
1826 induced by activational effects of androgens. Experimental studies testing the effects of
1827 androgens on the expression of ARTs were mainly conducted in fish and reptile species,
1828 and we report here the first field experiment on the role of testosterone in a mammalian
1829 species showing male ARTs.
1830 The African striped mouse, *Rhabdomys pumilio*, is a socially flexible species
1831 (Schradin et al. 2012b), that is, both males and females can change their reproductive
1832 tactics as a response to changing environmental conditions leading to changes in the
1833 social system. Three male ARTs have been identified that differ in steroid hormone levels
1834 (Schradin et al. 2009b) and in reproductive success (Schradin et al. 2012b): (i) philopatric
1835 group-living males with low testosterone and high corticosterone levels and very low
1836 reproductive success, (ii) solitary-living roamers with high testosterone and low
1837 corticosterone levels and intermediate reproductive success, (iii) dominant group-living
1838 territorial breeders with intermediate testosterone and low corticosterone levels and high
1839 reproductive success. Males that change their tactic also change their hormone profile.
1840 For example, philopatric group-living males that become solitary-living roamers have
1841 increased testosterone levels after the change in tactic (Schradin & Yuen 2011).
1842 Philopatric group-living males have to disperse from their natal group to become either
1843 solitary-living roamers or dominant group-living territorial breeders; they cannot become
1844 the breeding male in their natal group (Schradin et al. 2009b; Schradin & Lindholm
1845 2011). Males can disperse as juveniles at four weeks of age to become solitary-living
1846 roamers (Schradin 2005), or alternatively they become philopatric group-living males
1847 (Schradin et al. 2012b). Importantly, juvenile males do not directly become dominant
1848 group-living territorial breeders, because the latter are larger and more dominant than
1849 juvenile males (Schradin et al. 2009b; Schradin & Lindholm 2011). In sum, when
1850 juvenile males can undergo sexual maturation (four weeks old), they can either become
1851 philopatric group-living males with low testosterone levels or solitary-living roamers

1852 with high testosterone levels, indicating that the development into solitary-living roamers
1853 might be regulated by an increase in testosterone levels (Schradin & Yuen 2011).
1854 We examined the effects of increased testosterone levels on phenotypic traits in
1855 juvenile male striped mice. We focused on traits that differ between philopatric group-
1856 living males and solitary-living roamers. Compared to solitary-living roamers, philopatric
1857 group-living males are more often non-scrotal (proportion of non-scrotal philopatric
1858 group-living males: 28.84 % *vs.* proportion of non-scrotal solitary-living roamers: 0 %)
1859 (Schradin et al. 2009b), have smaller testes when being scrotal (Schradin et al. 2012a),
1860 and show higher corticosterone levels (Schradin et al. 2009b) due to environmentally-
1861 and socially–induced sexual suppression (Schradin et al. 2009a). High glucocorticoid
1862 levels appear to inhibit steroidogenesis of androgens, spermatogenesis and gonad
1863 development (Rivier & Rivest 1991). High testosterone levels can alter the hypothalamus
1864 pituitary adrenal (HPA) axis response to stress as demonstrated in male Sprague Dawley
1865 rats, *Rattus norvegicus*, (Viau 2002; Lund et al. 2004; Lund et al. 2006) and in men
1866 (Rubinow et al. 2005). Gonadal steroids such as testosterone can also modulate basal
1867 corticosterone levels (Kitay 1963). For example, in Sprague-Dawley male rats,
1868 gonadectomised males showed higher basal corticosterone levels than control males,
1869 while gonadectomised males with testosterone or dihydrotestosterone replacement
1870 showed a return to the basal corticosterone levels of intact males (Seale et al. 2004). This
1871 suggests that the interaction of the hypothalamus pituitary gonadal (HPG) axis with HPA
1872 might mediate gonadal functions (Handa et al. 1994; Viau 2002). In striped mice,
1873 increased testosterone levels could thus lead to decreased corticosterone secretion and the
1874 activation of the HPG axis, starting gonadal function. We thus predicted that juvenile
1875 males with testosterone implants would undergo sexual maturation indicated by lower
1876 basal corticosterone levels, larger testes, larger epididymides, and higher spermatogenesis
1877 activity than control males.
1878 A difference between philopatric and roaming African striped mouse males is that
1879 philopatrics are group-living while roamers are solitary-living. Furthermore, solitary-
1880 living roamers have larger home ranges than dominant group-living territorial breeders,
1881 which in turn have larger home ranges than philopatric group-living males (Schradin et
1882 al. 2010). Experimental increases of circulating androgen levels can lead to increased

home range sizes such as in side-blotched lizard males (*Uta stansburiana*) (Denardo & Sinervo 1994), Galapagos marine iguana males (*Amblyrhynchus cristatus*) (Wikelski et al. 2005), and bank voles (*Myodes glareolus*) (Mills et al. 2009). We thus predicted that juvenile males with testosterone implants would first expand their home ranges, searching for dispersal and mating opportunities, and then leave their natal group and start solitary living.

Materials and methods

Study area

Striped mice were studied during the breeding season 2010, from September to November. Our field site of 17 ha was located on the farm Klein Goegap (29°42.30'S - 18°02.95'E) in the Succulent Karoo of South Africa.

Trapping and marking of animals

Reproductive status and body mass were monitored by trapping striped mice directly at their nests by using metal live traps similar to Sherman's traps (26×9×9 cm) baited with a mixture of bran flakes and salad oil. Males were recorded as being either scrotal (testes fully descended) or non-scrotal. Mice were permanently marked using numbered metal ear tags (National Band and Tag Co., Newport, KY, USA). Additionally, each individual was dyed for visual identification with a mark on the pelage (Rapido, Pinetown South Africa). Group composition was determined both by direct nest observations at least one day a week and by radiotracking at night (see below). Nests were observed twice a day during the morning and evening for 30 min each, and the presence of all marked individuals was recorded (Schradin et al. 2007).

Experimental hormone manipulation:

Non-scrotal juvenile males were captured for study when they achieved a body mass of 19-30 g, at approximately four weeks old (Schradin et al. 2009b), the earliest age for dispersal (Schradin 2005). Study subjects were anaesthetized with di-ethyl ether and the subcutaneous implantation was performed behind the neck with a precision 10 gauge trochar (Innovative Research of America, Sarasota, FL, USA). They received a 4 mm

silastic implant (inner diameter 0.147 cm, outer diameter 0.196 cm) randomly containing either 0.2 mg of testosterone (Acros Organics, New Jersey, USA) or being empty. Ten juvenile males received the testosterone implant (testosterone-treated males) and eight others received an empty implant (control-treated males). Eight testosterone-treated males and six control-treated males originated from six groups with a maximum of two testosterone-treated males and one control-treated male per litter in a group. Two testosterone-treated males and two control-treated males originated from three solitary-living females with a maximum of one testosterone-treated male and one control-treated male per litter.

Determination of home ranges and sleeping sites:

All testosterone-treated and control-treated males were equipped with MD-2C radiotransmitters weighing 1.2 g (Holohil, Carp, Ontario, Canada). Radiotracking was performed with an AOR 8000 wide-range receiver (Tokyo, Japan) and an H-antenna (Africa Wildlife Tracking, Pretoria, South Africa). Home ranges were determined for eight non-consecutive days by radiotracking striped mice six times per day from 9:00 to 12:00 am and from 2:00 to 5:00 pm when striped mice are most active (Schradin & Pillay 2005). The accuracy of the GPS device (eTrex Venture, GARMIN International, Olathe, KS, USA) was ± 6 m at our field site. To calculate home range sizes, we used the convex polygon (100% cores) method with the RANGES6 software.

Composition of sleeping groups was determined by radiotracking at night when mice are inactive. For this, 10 breeding females (including the eight mothers of studied subjects) and 10 dominant group-living territorial breeders were also equipped with MD-2C radiotransmitters weighing 2 g (Holohil, Carp, Ontario, Canada). Individuals were considered as solitary-living when they slept alone for at least 70% of the time and group-living when they slept more than 50% of the time with at least one other adult individual (Schradin et al. 2010).

The duration of increased testosterone levels was set to 14 days because this time period was considered to be long enough to allow juvenile males to explore their environment and disperse. Juvenile philopatric striped mouse males typically increase

their home range for about one week before becoming solitary-living roamers (Schradin, unpubl. data).

Blood samples and tissue collection:

Blood samples were collected in the morning when individuals emerged from their nest between 7:00 and 8:00 am, controlling for possible circadian rhythms of hormone release. As soon as a striped mouse entered a trap, it was removed and anaesthetized with di-ethyl ether and a blood sample of about 200 µl was collected from the sub-lingual vein (Heimann 2006) within less than three minutes. After one hour, blood samples were centrifuged for 10 min (1000 rpm; Biofuge pico, Kendro Laboratory Products), the serum pipetted and centrifuged again to remove any blood cells, then frozen in aliquots of 50 µl for testosterone and of 10 µl for corticosterone assays.

For each testosterone-treated and control-treated male, we collected a first blood sample on D-1, i.e. the day before the implantation (D0). We collected a second and a third blood sample six days (D6) and 14 days (D14) after the implantation. Due to predation (for details see below "data analysis" section), we only collected a second blood sample for seven of the ten testosterone-treated males and six of the eight control-treated males. A third blood sample was obtained for five testosterone-treated males and six control-treated males. After the third blood sample, males were sacrificed by asphyxia to collect their testes and epididymides (five treated and six control), which were weighed and frozen at -20°C. For one of the testosterone-treated males, testes and epididymides were collected immediately after the second blood sample at the end of the field experiment.

Hormone assays:

For testosterone and corticosterone, we used commercial kits (IBL Hamburg, Germany) that had previously been validated for striped mouse serum (Schradin 2008). Since corticosterone levels are very high in philopatric group-living males (Schradin et al. 2009b), samples for the corticosterone assay were diluted 1:24. Eight out of 38 samples had a too small volume for testosterone measurements and had to be diluted with a zero standard. All 38 samples were analyzed within a single assay for testosterone and a single

assay for corticosterone. All samples were run in duplicate. The intra-assay coefficients of variation, calculated from the coefficients of variation from the samples measured in duplicate, were 2.95 % for testosterone and 7.66 % for corticosterone.

Sperm counts and cell cycle stages in testes

Testis and epididymis samples were analyzed at the Leibniz Institute for Zoo- and Wildlife Research (Berlin, Germany). For the determination of the number of testicular spermatozoa, testis parenchyma from the outer third of the testis (avoiding the rete testis region) was cut with a sharp razor blade into small pieces. Testis parenchyma was weighed, minced and suspended in 2 ml Dulbecco's buffered saline (Sigma D 8537) while carefully pressing through a 28 µm nylon mesh. After appropriate dilution in water, the sperm concentration was counted in a haemocytometer and given as the total sperm number.

For the analysis of cell cycle stages in the testes, frozen testis parenchyma was thawed, testicular cells were dispersed and flow cytometric DNA analysis was applied according to (Blottner et al. 1996): testis parenchyma was fine minced in 1 ml 100 mM citric acid containing 0.5% (v/v) Tween 20 and agitated for 20 min at room temperature. The DNA was stained by adding 4 ml of a 400 mM Na_2HPO_4 solution containing 5 µM 4',6-diamidino-2-phenylindol (DAPI) for 10 min in the dark. Measurements were performed on a PAS III flowcytometer (Partec, Germany) equipped with a mercury lamp (excitation: 360 nm, emission: 420 nm). Cells were counted and the histograms were analyzed for the proportions of cells in each peak by the FlowMax software (Partec, Germany). Haploid signals (1C) come from postmeiotic germ cell stages like spermatids and sperm cells, diploid signals (2C) from spermatogonia, secondary spermatocytes and somatic testicular cells, (4C) tetraploid signals mainly derive from G2/M phase of cell cycle in meiotic primary spermatocytes but also in mitotic spermatogonia. Between 2C and 4C, cells in the S-phase (S) were detected. Germ cell transformations were evaluated with the calculation of the diploid/tetraploid ratio (2C/4C), haploid/diploid ratio (1C/2C), and haploid/tetraploid ratio (1C/4C).

Data analysis:

Due to natural predation, samples sizes were reduced. Predation was ascertained with certainty. We found two radiotransmitters in snake feces (snake predators were daily radiotracked until radiotransmitters were recovered) and we found one fresh dead male with a snake bite to the body. We found one radiotransmitter on the ground close to a male's nest (the wire mesh of the transmitter was found intact, indicating that this male did not loose its transmitter but was eaten). One male got a signal from its transmitter coming from the crack of a small hill, indicating that this male was taken by a bird of prey. Three testosterone- and two control-treated males were predated before the second blood sample. One testosterone-treated male was predated before the third blood sample. Thus, data from testosterone-treated males and control-treated males were considered for statistical analyses when we could collect at least a second blood sample for these males.

Home range data were available for six testosterone-treated males and six control-treated males. The maximum of available days of radiotracking were (t: test males / c: control males): eight days (t: 2 / c: 4), seven days (t: 1), five days (t: 1 / c: 2), four days (t: 2). For males with less than 8 days of radio-tracking data, we calculated expected home ranges for day 8. For this, we multiplied home-range size on the last day of radiotracking by the percentage by which home ranges increased from the last day of radiotracking to 5, 6, 7, and 8 days of radiotracking in the remaining males of the study (for the same approach see (Schradin & Pillay 2005)). Thereafter, we calculated slopes and intercepts of the relationships between home ranges and days of radiotracking for each testosterone-treated and control-treated male before and after the home range corrections using linear regression functions. We therefore showed that there was no significant difference in slope values before ($mean_{slope}$=0.06±0.05) and after home range corrections ($mean_{slope}$=0.05±0.03) (exact Wilcoxon signed rank test: N=12; V=15; p=0.70). Additionally and for comparison, we obtained home range data for seven of the ten breeding males equipped with radio-collars.

Statistical analyses were carried out with R 2.12.2 (R Development Core Team 2011). Results are presented as mean ± SD and significance was accepted at $\alpha \leq 0.05$. We used non-parametric statistical analyses due to small sample sizes. Pairwise or multiple comparisons between D-1, D6, and D14 within an experimental group (i.e. testosterone-

2037 treated or control-treated males) were performed with paired exact Wilcoxon signed rank
2038 tests and Friedman rank sum tests. Comparisons between home ranges and body mass of
2039 dominant group-living territorial breeders with testosterone-treated males and with
2040 control-treated males were performed with unpaired Exact Wilcoxon Rank Sum Tests
2041 (equivalent to Mann Whitney U test with R2.12.2). Comparisons between testosterone-
2042 treated and control-treated males were also performed with unpaired Exact Wilcoxon
2043 Rank Sum Tests and Fisher's Exact Tests. To test for a relationship of testosterone levels
2044 with corticosterone levels, we performed a Spearman correlation. For these correlations,
2045 data for testosterone-treated and control-treated males were combined.
2046 To test for the effect of the treatment on the testis and epididymis weights we used
2047 two Generalized Linear Models (GLM) fitted with a quasi binomial error distribution
2048 (N=12; 6 testosterone-treated and 6 control-treated males). Testis mass was modeled
2049 following a proportion data analysis (Crawley 2007) by binding together the vectors of
2050 testis mass and body mass (i.e., male body mass corresponding to the day of testis
2051 collection) into a single object considered as a response. The response variable was also
2052 squared root transformed to achieve linearity of residuals. Epididymis mass was analyzed
2053 following the same procedure for testis mass. Finally, the effect of the treatment on
2054 whether testosterone-treated and control-treated males slept alone was analyzed with
2055 Generalized Linear Models (GLM) fitted with a quasi binomial error distribution (N=12;
2056 6 test and 6 control males). The number of times test and control males were sleeping
2057 with another member in their group were modeled with a proportion data analysis by
2058 binding together the vector of the number of times testosterone-treated and control-
2059 treated males sleept alone and the vector of the total number of times testosterone-treated
2060 and control-treated males were radiotracked for sleeping site determination into a single
2061 object considered as a response.
2062
2063 **Results**
2064 *Description of the study population*
2065 Nine groups (with 14 females born the previous breeding season) and five solitary
2066 breeding females were present on our field site during the experiment. The ratio of
2067 solitary- to group-living females was 0.36. One of these 19 adult females produced two

litters, and seven adult females produced a single litter, while eleven adult females (58%) did not reproduce at all. All ten adult males were scrotal and nine of these males were dominant group-living territorial breeders while one male followed a roaming tactic. We only observed one adult philopatric male that was group-living at the beginning of the breeding season.

Testosterone levels in juvenile males

Testosterone-treated and control-treated males showed similar testosterone levels before the treatment on D-1 (0.42±0.59 ng/ml *vs.* 0.18 +0.27 ng/ml; W=27; p=0.42; Figure 1). Testosterone-treated males had significantly higher testosterone levels than control-treated males on D6 (8.32+4.45 ng/ml *vs.* 0.28+0.33 ng/ml; W=42; p=0.003) and on D14 (1.58+1.14 ng/ml *vs.* 0.14+0.22 ng/ml; W=30; p=0.006). We did not observe significant changes of testosterone levels among control-treated males during the experiment (Friedman chi-squared=6.07; p=0.30). Testosterone levels of testosterone-treated males increased significantly from D-1 to D6 (V=0; p=0.02), then decreased from D6 to D14 though not quite significantly (V=15; p=0.06; Figure 1). The testosterone levels of testosterone-treated males did not increase significantly from D-1 to D14 (V=3, p=0.31).

Corticosterone levels in juvenile males

Testosterone-treated and control-treated males showed similar corticosterone levels before the treatment on D-1 (758.16±394.41 ng/ml *vs.* 832.19±524.73 ng/ml; W=19; p=0.84; Figure 2). Testosterone-treated males had significantly lower corticosterone levels than control-treated males on D6 (649.45±212.80 ng/ml *vs.* 1082.38±268.28 ng/ml; W=4; p=0.01) but not on D14 (409.85±157.15 ng/ml *vs.* 683.14.85±463.29 ng/ml; W=11; p=0.54). We did not observe significant changes of corticosterone levels among control-treated males during the experiment (Friedman chi-squared=6.24; p=0.28). Corticosterone levels of testosterone-treated males did not decrease significantly from D-1 to D6 (V=17; p=0.69) nor from D6 to D14 (V=10; p=0.63). The corticosterone levels of testosterone-treated males decreased, though not quite significantly, from D-1 to D14 (V=15, p=0.06).

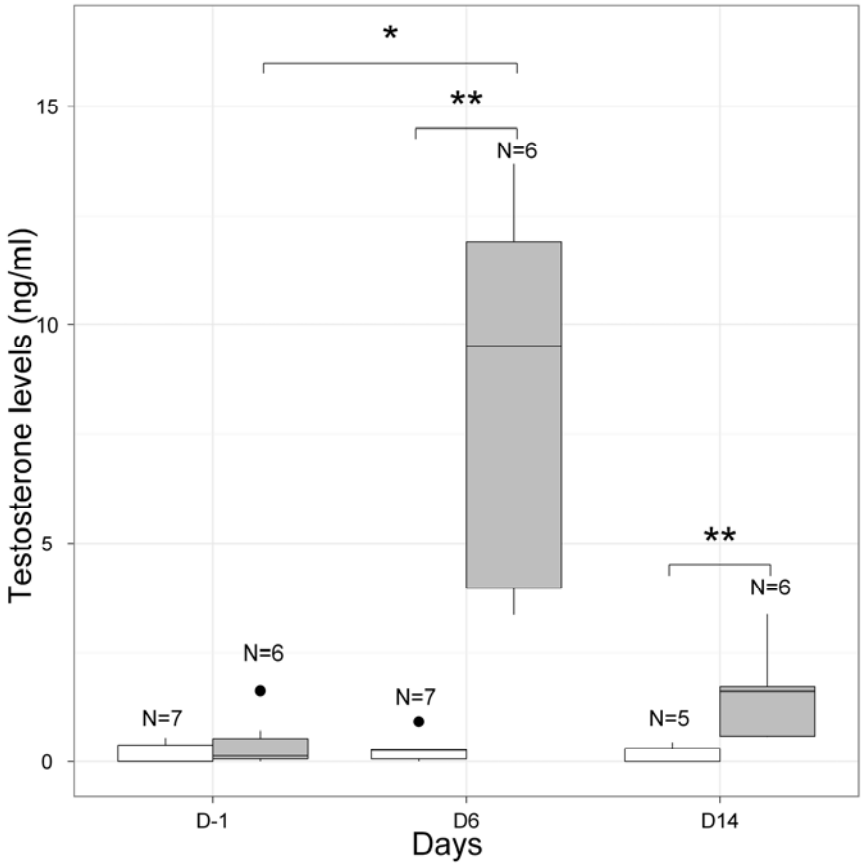

Figure 1. Serum testosterone levels before (D-1), six days (D6), and 14 days (D14) after the testosterone treatment in testosterone- (grey) and control-treated males (white). The median is indicated by the horizontal bar inside the box, the first and third quartile by the box itself, and the horizontal bars outside the box indicate the minimal values and the fourth quartile (outliers are shown by a black-filled circle). Only significant pairwise comparisons are reported on the figure: *****: $p<0.05$; ******: $p<0.01$.

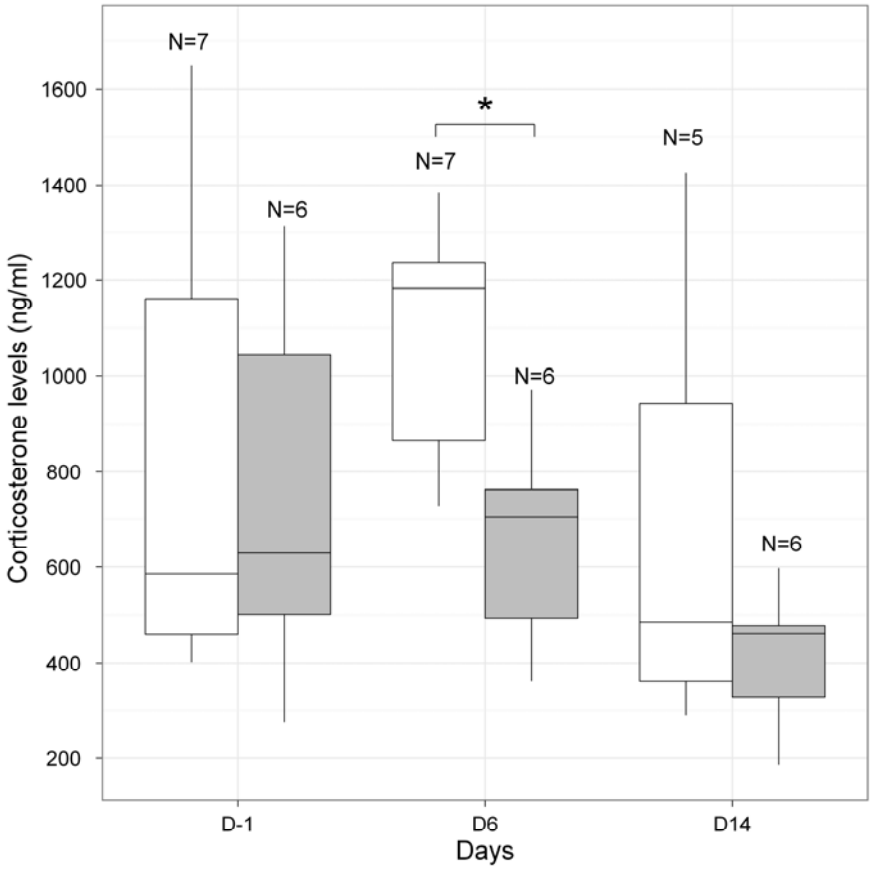

Figure 2. Serum corticosterone levels before (D-1), six days (D6), and 14 days (D14) after the testosterone treatment in testosterone- (grey) and control-treated males (white). The median is indicated by the horizontal bar inside the box, the first and third quartile by the box itself, and the horizontal bars outside the box indicate the minimal values and the fourth quartile. Only significant pairwise comparisons are reported on the figure: *****: $p<0.05$.

Correlations between testosterone and corticosterone levels in juvenile males

Testosterone and corticosterone levels were significantly negatively correlated at D6 (r_s=-0.58; p=0.038) but not at D-1 (r_s=-0.16; p=0.59) and D14 (r_s=-0.31; p=0.35).

Body mass

Testosterone-treated and control-treated males did not differ significantly in body mass, neither before (W=19; p=0.83) nor after the treatment (D6: W=24; p=0.72; D14: W=20.5;

p=0.36; Table 1). The mean body mass of dominant group-living territorial breeders was two times greater than the body mass of testosterone-treated males (53.97±3.39g vs. 25.71±3.25g; W=49; p<0.001) and of control-treated males (53.97±3.39g vs. 25.00±4.56g; W=42; p<0.01) at the end of their testosterone or control treatment.

Reproductive parameters of juvenile males

All males were non-scrotal on D-1. On D6, two out of seven testosterone-treated males and none of the six control-treated males were scrotal (Fisher's Exact Test: p=0.46). At D14, all testosterone-treated males but none of the six control-treated males were scrotal (Fisher's Exact Test: p=0.002).

Testosterone treatment was significantly associated with testis mass (GLM: $F_{1,10}$=19.58; p=0.001) and with epididymis mass (GLM: $F_{1,10}$=116.36; p<0.001), both being heavier in testosterone-treated males (Table 1).

Table 1. Body mass of the testosterone- and control-treated males at D-1, D6, and D14, and testis and epididymis mass presented as the percentage of the body mass of the testosterone- and control-treated males. Mean ± SD.

	Body mass (g)			Testis (%)	Epididymis (%)
	D-1	D6	D14		
Test males	22.0±3.6 (N=7)	23.7±3.4 (N=7)	25.8±3.1 (N=5)	0.26±0.04 (N=6)	0.044±0.005 (N=6)
Control males	22.3±4.1 (N=6)	23.0±4.9 (N=6)	25.0±4.6 (N=6)	0.08±0.02 (N=6)	0.012±0.002 (N=6)

Testes of testosterone-treated males had, though not quite significantly, greater haploid signals (1C: W=32.5; p=0.06), significantly lower diploid signals (2C: W=2; p=0.009) significantly greater tetraploid signals (4C: W=34; p=0.009), and more cells in the S-phase (S: W=31; p=0.04) than testis of control-treated males (Table 2). The haploid/diploid ratio (1C/2C) was higher, though not significantly, for the testis of testosterone-treated males than those of control-treated males (W=29; p=0.08). No significant difference was observed for the haploid/tetraploid ratio (1C/4C) (W=24;

p=0.34). We found a significantly lower diploid/tetraploid ratio (2C/4C) in the testis of testosterone-treated males than of control-treated males (W=2; p=0.009). Spermatozoa were only found in the testis of a single testosterone-treated male. This male was the only male with a 1C/4C ratio greater than one (1.6).

Table 2. Ploidy-state of the testis tissues of testosterone- and control-treated males. %1C, %2C, %4C, and %S are the percentages of haploid, diploid, and tetraploid signals as well as cells in the S-phase. 2C/4C, 1C/4C, and 1C/2C are the signal ratios. Mean ± SD.

Signals and ratios	Test males (N=6)	Control males (N=6)
%1C	9.56±12.38	0.57±0.36
%2C	56.23±26.01	84.98±4.50
%S	9.73± .90	6.29±1.18
%4C	22.78±12.40	6.87±3.44
2C/4C	4.12±4.52	14.85±6.22
1C/4C	0.40±0.60	0.09±0.06
1C/2C	0.39±0.60	0.01±0.00

Home ranges and sleeping sites

Testosterone-treated males had on average twice as large home ranges as control-treated males (0.60±0.25 ha *vs.* 0.28±0.08 ha; W=31; p=0.045) (Figure 3). Home range sizes of testosterone-treated males did not differ significantly from those of dominant group-living territorial breeders (0.60±0.25 ha *vs.* 0.75±0.87ha; W=25; p=0.62). Although they were about one third the size, home range sizes of control-treated males did not differ significantly from those of dominant group-living territorial breeders (0.28±0.08 ha *vs.* 0.75±0.87ha; W=11.5; p=0.20).

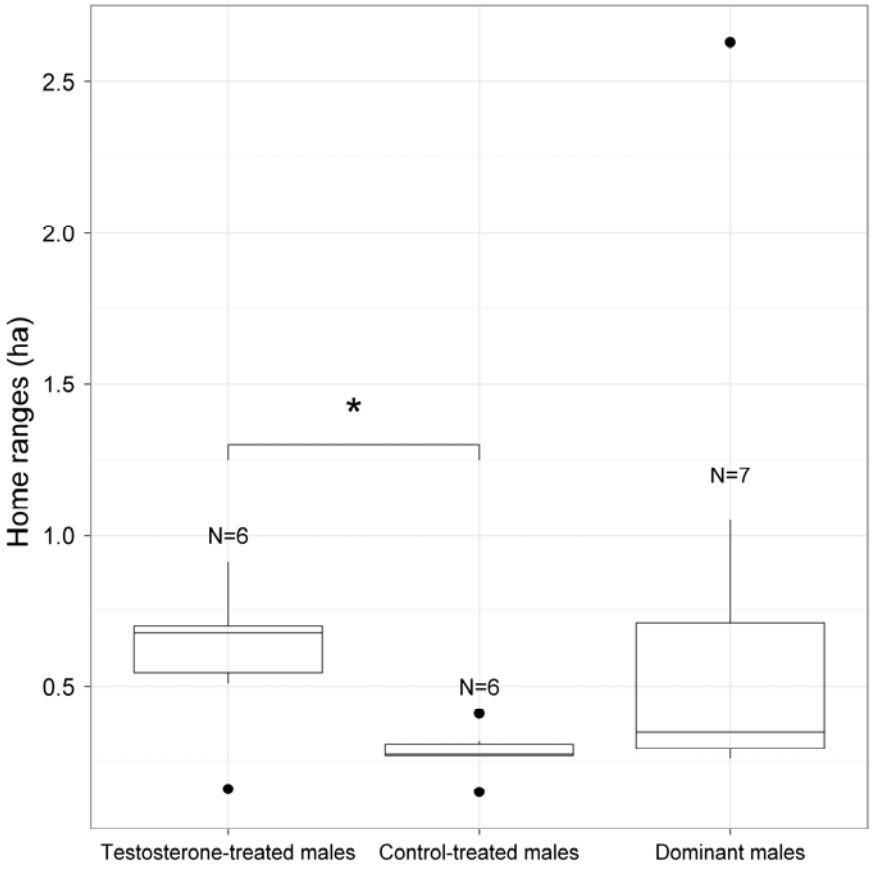

Figure 3. Home range sizes (mean ± SD) after eight days of radiotracking for testosterone-treated males (grey), control-treated males (white), and dominant group-living territorial breeders (black). The median is indicated by the horizontal bar inside the box, the first and third quartile by the box itself, and the horizontal bars outside the box indicate the minimal values and the fourth quartile (outliers are shown by a black-filled circle). Only significant pairwise comparisons are reported on the figure: *****: $p<0.05$.

Both testosterone-treated and control-treated males slept most of the time with another member of the group between D-1 and D14 (93.55±2.17% *vs.* 98.39±1.02%; GLM: $F_{1,11}=2.87$; $p=0.12$).

Discussion

To date, few studies have demonstrated that an experimental increase of circulating testosterone levels can influence which reproductive tactic an individual will follow or

2180 that it can accelerate the development of a specific reproductive tactic (Oliveira et al.
2181 2008). According to the high testosterone levels observed in solitary-living roamers
2182 (Schradin et al. 2009b; Schradin & Yuen 2011), we predicted that juvenile males with an
2183 experimentally-induced increase in serum testosterone levels during the breeding season
2184 would undergo morphological, physiological, and behavioral changes indicating the onset
2185 of puberty. We observed that testosterone implants led to changes in corticosterone
2186 levels, accelerated testes development, activated spermatogenesis, and increased home
2187 range sizes. Testosterone-treated males had no opportunity to become the dominant
2188 group-living territorial breeder of another group, as their body mass was only 50% of the
2189 dominant breeders. Importantly, body mass determines competitive ability and thus
2190 reproductive tactics in African striped mice (Schradin et al. 2009b; Schradin & Lindholm
2191 2011; Schradin et al. 2012b). However, they could have left their natal group and become
2192 solitary living roamers, which they did not, indicating that the onset of the roaming tactic
2193 was incomplete.

2194 Male puberty occurs when gonadotropin-releasing hormone and gonadotropin
2195 secretion increases (Banerjee & Clayton 2007) resulting in gonadal testosterone surges,
2196 which leads to sexual maturation (Nelson 2005). Juvenile males with an experimental
2197 increase in serum testosterone levels became scrotal and had heavier testes and
2198 epididymides than the non-scrotal control-treated males. This demonstrates that our
2199 testosterone treatment induced sexual maturation in juvenile males. Furthermore, we
2200 found evidence that an increase in circulating testosterone levels activated
2201 spermatogenesis in juvenile males. This is in agreement with the general pattern in male
2202 vertebrates, where increased circulating testosterone levels are known to enhance the
2203 development of external (i.e. scrotal development) and internal reproductive structures
2204 (i.e. testicular development), as well as spermatogenesis (Nelson 2005).

2205 Spermatogenesis can be studied by measuring the DNA content of cells in the
2206 testes (Blottner et al. 1996). Haploid signals (1C) originating from round spermatids or
2207 spermatozoa were observed more often in the testes of testosterone-treated males than
2208 those of control-treated males, which showed almost no haploid signals. But spermatozoa
2209 were microscopically observed in only one test male. This juvenile male was the only one
2210 with a ratio of haploid to tetraploid signals greater than one, which is a measure of the

meiotic yield from primary spermatocytes to the haploid product. Since the duration of spermatogenesis in laboratory mice (*Mus musculus domesticus)* is about 35 days (Clermont 1972), an experimental increase of plasma testosterone levels of 15 days might have been too short to lead to complete spermatogenesis in testosterone-treated males. Importantly, the meiotic and/or mitotic activity was greater in the testes of testosterone-treated males than of control-treated males (inferred from lower diploid/tetraploid ratio in testosterone-treated males). A high proportion of haploid nuclei indicate meiotic activity and the onset of sperm production, at least to the stage of round spermatids. This was indicated by a higher haploid/diploid ratio in test males. Both results are indicative of the occurrence of meiosis in testosterone-treated males but not in control-treated males. We conclude that spermatogenetic activity began in the testes of testosterone-treated males but not of control-treated males.

In African striped mice, juvenile male kept individually in captive condition are typically sexually mature (scrotal) when being 3 - 4 weeks old (Schradin et al. 2009a; Schradin et al. 2012a). In contrast, juvenile males kept in family group are sexually mature when being 4 - 5 weeks old (the age of juvenile males used in the present study at the start of our testosterone treatment) (Schradin et al. 2009a; Schradin et al. 2012a). In fact, juvenile males remaining in their family group delay sexual maturation (Schradin & Pillay 2004) due to environmentally- and socially–induced sexual suppression (Schradin et al. 2009a). High corticosterone levels might be the endocrine mechanism of this suppression (Schradin et al. 2009b). Interestingly, testosterone-treated males showed lower basal corticosterone levels than control-treated males after (but not before) the treatment. Central and peripheral actions of testosterone leading to decreased glucocorticoid levels have been described in several previous studies (Viau & Meaney 1996; Lund et al. 2004; Rubinow et al. 2005; Lund et al. 2006). Thus, the testosterone treatment had likely suppressive effects on the activity of the HPA axis. The resulting decrease of corticosterone secretion from the adrenal gland might have lifted the inhibition of gonadal functions, especially spermatogenesis, in testosterone-treated males, but not in control-treated males. Our results indicate that an interaction between testosterone and corticosterone might regulate the reproductive capacity of juvenile males (Rivier & Rivest 1991). Taken together, the faster gonad development and the activation

of gonadal function induced by the increase of testosterone levels demonstrate that testosterone-treated males underwent sexual maturation, which was inhibited or at least delayed in control-treated males.

In the present study, the silastic testosterone implants increased testosterone levels to the upper physiological range of testosterone observed in solitary-living roamers (9 ng/ml in (Schradin & Yuen 2011)). Experimental increase of testosterone levels led to significant changes in the physiological and reproductive status of juvenile males. However, testosterone-treated males remained as philopatric group-living males, just as control males. Why did testosterone-treated males not leave their natal group to become solitary-living roamers? We observed that testosterone-treated males had larger home ranges than control males. Testosterone can reduce anxiety in laboratory male mice (Aikey et al. 2002) and it is known from other species that experimental testosterone administration can lead to larger home range sizes (Chandler et al. 1994; Denardo & Sinervo 1994; Wikelski et al. 2005; Mills et al. 2009). Increasing home range size might help individuals to gather information about when and where to disperse. However, the decision to disperse and to become solitary-living roamers or to stay in the natal group as philopatric group-living males may also depend on environmental conditions. During our experiment, five out of 19 adult females were breeding solitarily and most adult males were dominant group-living territorial breeders. Schradin and Lindholm (2011) predicted that philopatric group living males would make the decision to remain group-living philopatric males and not to disperse when the ratio of solitary- to group-living females is below 1, as it was in our study. Furthermore, under normal environmental conditions 100% of females reproduce during the breeding season (Schradin & Lindholm 2011), but extremely low rainfall during our study resulting in low food availability might have been the reason why only 42% of females reproduced. This limited the chances for solitary-living roamers to reproduce, and might explain why only one solitary-living roamer was present in the population. The specific environmental conditions of our field study, i.e. the few mating opportunities, could explain why testosterone-treated juvenile males did not disperse and did not become solitary-living roamers. In sum, increased testosterone and decreased corticosterone levels were sufficient to induce sexual maturation, but were not sufficient to elicit the development into the solitary-living tactic.

Conclusion

To our knowledge, this is the first study experimentally testing the activational effects of testosterone on the induction of sexual maturation and the onset of ARTs in a free-ranging mammal species. We observed that the experimental increase of testosterone levels accelerated sexual maturation but did not lead to dispersal and solitary-living. The larger home ranges of testosterone-treated males could be due to testosterone-induced anxiolytic effects (Aikey et al. 2002), allowing testosterone-treated males to assess the prevailing environmental conditions. However, the environmental conditions of our study were not favorable for dispersal, as reproductive opportunities were few (Schradin & Lindholm 2011). This could explain why juvenile males hormonally primed to reproduce did not become solitary-living roamers. Therefore, we recommend that future studies consider the role of environmental cues in addition to hormonal signals in the neuroendocrine control of ARTs.

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References

Aikey, J. L., Nyby, J. G., Anmuth, D. M. & James, P. J. 2002. Testosterone rapidly reduces anxiety in male house mice (Mus musculus). *Hormones and Behavior*, **42**, 448-460.

Arnold, A. P. & Breedlove, S. M. 1985. Organizational and activational effects of sex steroids on brain and behavior - a reanalysis. *Hormones and Behavior*, **19**, 469-498.

Banerjee, I. & Clayton, P. 2007. The genetic basis for the timing of human puberty. *Journal of Neuroendocrinology*, **19**, 831-838.

Beach, F. A. 1975. Hormonal modification of sexually dimorphic behavior. *Psychoneuroendocrinology*, **1**, 3-23.

Blottner, S., Hingst, O. & Meyer, H. H. D. 1996. Seasonal spermatogenesis and testosterone production in roe deer (Capreolus capreolus). *Journal of Reproduction and Fertility*, **108**, 299-305.

Chandler, C. R., Ketterson, E. D., Nolan, V. & Ziegenfus, C. 1994. Effects of testosterone on spatial activity in free-ranging male dark-eyed juncos, junco-hyemalis. *Animal Behaviour*, **47**, 1445-1455.

Clermont, Y. 1972. Kinetics of spermatogenesis in mammals - seminiferous epithelium cycle and spermatogonial renewal. *Physiological Reviews*, **52**, 198-236.

Crawley, M. 2007. *Statistics An Introduction using R*. West Sussex: Wiley.

de la Cruz, C., Solis, E., Valencia, J., Chastel, O. & Sorci, G. 2003. Testosterone and helping behavior in the azure-winged magpie (Cyanopica cyanus): natural covariation and an experimental test. *Behavioral Ecology and Sociobiology*, **55**, 103-111.

Denardo, D. F. & Sinervo, B. 1994. Effects of steroid-hormone interaction on activity and home-range size of male lizards. *Hormones and Behavior*, **28**, 273-287.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: Diversity within sexes. *Trends in Ecology & Evolution*, **11**, 92-98.

Handa, R. J., Burgess, L. H., Kerr, J. E. & Okeefe, J. A. 1994. Gonadal-steroid hormone receptors and sex-differences in the hypothalamo-pituitary-adrenal axis. *Hormones and Behavior*, **28**, 464-476.

Heimann, M. 2006. Development and validation of the method of sublingual blood sampling in mice and other small rodents, University of Zurich.

Hews, D. K. & Moore, M. C. 1996. A critical period for the organization of alternative male phenotypes of tree lizards by exogenous testosterone? *Physiology & Behavior*, **60**, 425-429.

Hews, D. K., Knapp, R. & Moore, M. C. 1994. Early exposure to androgens affects adult expression of alternative male types in tree lizards. *Hormones and Behavior*, **28**, 96-115.

Kitay, J. I. 1963. Pituitary-adrenal function in rat after gonadectomy and gonadal hormone replacement. *Endocrinology*, **73**, 253-260.

Lund, T. D., Hinds, L. R. & Handa, R. J. 2006. The androgen 5 alpha-dihydrotestosterone and its metabolite 5 alpha-androstan-3 beta,17 beta-diol inhibit the hypothalamo pituitary-adrenal response to stress by acting through estrogen receptor beta-expressing neurons in the hypothalamus. *Journal of Neuroscience*, **26**, 1448-1456.

Lund, T. D., Munson, D. J., Haldy, M. E. & Handa, R. J. 2004. Dihydrotestosterone may inhibit hypothalamo-pituitary-adrenal activity by acting through estrogen receptor in the male mouse. *Neuroscience Letters*, **365**, 43-47.

Mills, S. C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T., Oksanen, T. A. & Poikonen, T. 2009. Testosterone-Mediated Effects on Fitness-Related Phenotypic Traits and Fitness. *American Naturalist*, **173**, 475-487.

Moore, M. C. 1991. Application of organizational activation theory to alternative male reproductive strategies - a review. *Hormones and Behavior*, **25**, 154-179.

Moore, M. C., Hews, D. K. & Knapp, R. 1998. Hormonal control and evolution of alternative male phenotypes: Generalizations of models for sexual differentiation. *American Zoologist*, **38**, 133-151.

Nelson, R. J. 2005. *An Introduction to BEHAVIORAL ENDOCRINOLOGY*, Third edn. Sunderland: Sinauer Associates, INC.

Oliveira, R. F., Canário, A. V. M. & Ros, A. F. H. 2008. Hormones and alternative reproductive tactics in vertebrates. In: *Alternative Reproductive Tactics* (Ed. by R. F. Oliveira, M. Taborsky & H. J. Brockmann), pp. 132-173. Cambridge: Cambridge University Press.

2364 **Oliveira, R. F., Carneiro, L. A., Canario, A. V. M. & Grober, M. S.** 2001a. Effects of
2365 androgens on social behavior and morphology of alternative reproductive males of the
2366 azorean rock-pool blenny. *Hormones and Behavior*, **39**, 157-166.
2367 **Oliveira, R. F., Carneiro, L. A., Goncalves, D. M., Canario, A. V. M. & Grober, M.**
2368 **S.** 2001b. 11-ketotestosterone inhibits the alternative mating tactic in sneaker males of the
2369 peacock blenny, Salaria pavo. *Brain Behavior and Evolution*, **58**, 28-37.
2370 **Peters, A., Cockburn, A. & Cunningham, R.** 2002. Testosterone treatment suppresses
2371 paternal care in superb fairy-wrens, Malurus cyaneus, despite their concurrent investment
2372 in courtship. *Behavioral Ecology and Sociobiology*, **51**, 538-547.
2373 **Phoenix, C. H., Goy, R. W., Gerall, A. A. & Young, W. C.** 1959. Organizing action of
2374 prenatally administrated tetosterone propionate on the tissues mediating mating behaviour
2375 in the female guinea pig. *Endocrinology*, **65**, 369-382.
2376 **R Development Core Team**. 2011. R: A language and environment for statistical
2377 computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-
2378 0, URL http://www.R-project.org/.
2379 **Rivier, C. & Rivest, S.** 1991. Effect of stress on the activity of the hypothalamic-
2380 pituitary-gonadal axis - peripheral and central mechanisms. *Biology of Reproduction*, **45**,
2381 523-532.
2382 **Rubinow, D. R., Roca, C. A., Schmidt, P. J., Danaceau, M. A., Putnam, K., Cizza,**
2383 **G., Chrousos, G. & Nieman, L.** 2005. Testosterone suppression of CRH-stimulated
2384 cortisol in men. *Neuropsychopharmacology*, **30**, 1906-1912.
2385 **Schradin, C.** 2008. Seasonal changes in testosterone and corticosterone levels in four
2386 social classes of a desert dwelling sociable rodent. *Hormones and Behavior*, **53**, 573-579.
2387 **Schradin, C.** 2005. When to live alone and when to live in groups : ecological
2388 determinants of sociality in the African striped mouse (Rhabdomys pumilio, Sparrman,
2389 1784). *Belgian Journal of Zoology*, **135**, 77-82.
2390 **Schradin, C. & Lindholm, A. K.** 2011. Relative fitness of alternative male reproductive
2391 tactics in a mammal varies between years. *Journal of Animal Ecology*, **80**, 908-917.
2392 **Schradin, C. & Yuen, C.-H.** 2011. Hormone levels of male African striped mice change
2393 as they switch between alternative reproductive tactics. *Hormones and Behavior*, **60**, 676-
2394 680.

Schradin, C. & Pillay, N. 2005. Intraspecific variation in the spatial and social organization of the African striped mouse. *Journal of Mammalogy*, **86**, 99-107.

Schradin, C. & Pillay, N. 2004. The striped mouse (Rhabdomys pumilio) from the succulent karoo, South Africa: A territorial group-living solitary forager with communal breeding and helpers at the nest. *Journal of Comparative Psychology*, **118**, 37-47.

Schradin, C., Eder, S. & Müller, K. 2012a. Differential investment into testes and sperm production in alternative male reproductive tactics of the African striped mouse (Rhabdomys pumilio). *Hormones and Behavior*, **61**, 686-695.

Schradin, C., König, B. & Pillay, N. 2010. Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515-521.

Schradin, C., Schneider, C. & Yuen, C. H. 2009a. Age at puberty in male African striped mice: the impact of food, population density and the presence of the father. *Functional Ecology*, **23**, 1004-1013.

Schradin, C., Scantlebury, M., Pillay, N. & Koenig, B. 2009b. Testosterone Levels in Dominant Sociable Males Are Lower than in Solitary Roamers: Physiological Differences between Three Male Reproductive Tactics in a Sociably Flexible Mammal. *American Naturalist*, **173**, 376-388.

Schradin, C., Krackow, S., Schubert, M., Keller, C., Schradin, B. & Pillay, N. 2007. Regulation of activity in desert-living striped mice: The importance of basking. *Ethology*, **113**, 606-614.

Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., Koenig, B. & Pillay, N. 2012b. Social flexibility and social evolution in mammals: a case study of the African striped mouse (Rhabdomys pumilio). *Molecular Ecology*, **21**, 541-553.

Seale, J. V., Wood, S. A., Atkinson, H. C., Harbuz, M. S. & Lightman, S. L. 2004. Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. *Journal of Neuroendocrinology*, **16**, 989-998.

Sinervo, B., Miles, D. B., Frankino, W. A., Klukowski, M. & DeNardo, D. F. 2000. Testosterone, endurance, and darwinian fitness: Natural and sexual selection on the

physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior*, **38**, 222-233.

Sluyter, F., Bult, A., Lynch, C. B., Vanoortmerssen, G. A. & Koolhaas, J. M. 1995. A comparison between house mouse lines selected for attack latency or nest-building - evidence for a genetic-basis of alternative behavioral strategies. *Behavior Genetics*, **25**, 247-252.

Stevenson, I. R. & Bancroft, D. R. 1995. Fluctuating trade-offs favour precocial maturity in male Soay sheep. *Proceedings of the Royal Society B-Biological Sciences*, **262**, 267-275.

van Oortmerssen, G. 1971. Biological significance, genetics and evolutionary origin of variability in behaviour within and between inbred strains of mice (mus-musculus) - behaviour genetic study. *Behaviour*, **38**, 1-&.

Viau, V. 2002. Functional cross-talk between the hypothalamic-pituitary-gonadal and - adrenal axes. *Journal of Neuroendocrinology*, **14**, 506-513.

Viau, V. & Meaney, M. J. 1996. The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. *Journal of Neuroscience*, **16**, 1866-1876.

Wikelski, M., Steiger, S. S., Gall, B. & Nelson, K. N. 2005. Sex, drugs and mating role: testosterone-induced phenotype-switching in Galapagos marine iguanas. *Behavioral Ecology*, **16**, 260-268.

Wingfield, J. C., Hegner, R. E., Dufty, A. M. & Ball, G. F. 1990. The challenge hypothesis - theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *American Naturalist*, **136**, 829-846.

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Chapter 4

Regulation of male prolactin levels in an opportunistic breeding species, the African striped mouse

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Regulation of male prolactin levels in an opportunistic breeding species, the African striped mouse

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Abstract

The timing of reproduction of many species depends on seasonal changes in prolactin secretion. Photoperiod coincides with annual seasonal changes and typically regulates prolactin secretion. However, when environmental conditions are unpredictable, other ecological factors may contribute to prolactin regulation. In African striped mice (*Rhabdomys pumilio*), males show seasonal changes in reproduction and in prolactin levels, but unexpected increases of food availability out of the regular breeding season can also induce reproduction. We measured prolactin levels in free ranging African striped mouse males during three periods: 1. the natural breeding in spring with increasing photoperiod. 2. The natural non-breeding season in summer (dry season) with decreasing photoperiod. 3. During two summers with unexpected rainfall inducing breeding in the population. Here we report that breeding males showed increased prolactin levels when they were breeding independently of increases and decreases in day-length. Also, we found a positive correlation ($p=0.05$) between the availability of food plants and prolactin levels. Changes in prolactin levels in opportunistically breeding species like the African striped mouse are not strictly regulated by photoperiod, but seem to respond to cues from food availability.

Keywords: reproduction; social flexibility; testosterone; paternal care

Introduction

Prolactin is well known for its essential role in the reproduction of males and females (Nelson 2005). Many species show increased prolactin secretion during the breeding season (Curlewis 1992). Photoperiod typically regulates this hormone secretion (Goldman *et al.* 2008), i.e. the ratio of hours with daylight to the hours of darkness; and whether this ratio is currently increasing (i.e. winter to mid summer) or decreasing: prolactin levels increase when day length increases which coincides with reproductive periods in long-day breeders (Hall et al. 1986; Curlewis 1992; Sharp et al. 1998; Johnston 2004; Sharp 2005; Paul et al. 2008). For instance, experimental decrease of day length resulted in a decrease in blood prolactin levels in prairie voles, *Microtus orchrogaster* (Smale *et al.* 1988), golden hamster, *Mesocricetus auratus* (Steger *et al.* 1983), and Soay rams, *Ovis aries* (Lincoln *et al.* 1978). These changes in prolactin secretion mediate necessary physiological and behavioural changes between seasons (Wingfield 2008). Up-regulation of prolactin secretion can activate reproduction by stimulating stimulating follicule-stimulating hormone (FSH) release (Steger et al. 1983) and reducing sensitivity of gonadotropin release to negative testosterone feedback which is essential for annual reactivation of gonadal activity (Bartke 2004). High prolactin levels are also associated with parental care in birds and mammals (Buntin 1996; Schradin & Anzenberger 1999).

The photoperiodic regulation of prolactin is advantageous to time physiological and behavioural changes related to reproduction (Curlewis 1992). In predictable environments, increased day-length predicts when environmental conditions (food, water, and temperature) are optimal for breeding. Thus, the increase of prolactin secretion can occur in advance enabling an optimal start of reproduction (Wingfield 2008). If changes in environmental conditions are, however, unpredictable, photoperiodic cues cannot indicate when future necessary resources for breeding will be available. Under these environmental conditions, food, water, and temperature may play a more important role in the regulation of prolactin secretion (Dawson 2008). The zebra finch, *Taeniopygia guttata*, an opportunistic breeding species living in arid habitat, has increased prolactin levels outside of the breeding season, when food and water availability favour reproduction (Christensen & Vleck 2008). Similarly in California mice, *Peromyscus californicus*, experimental changes in day length and food availability did not influence prolactin levels but water availability did (Nelson *et al.* 1995). Such studies, nevertheless, are scarce and which environmental factors modulate prolactin secretion in opportunistically breeding species is still unclear.

The African striped mouse, *Rhabdomys pumilio*, is a seasonally breeding species with geographic variations in South Africa: breeding occurs in summer for 4-6 months in summer-rainfall areas, breeding occurs in spring for 3-4 months in winter-rainfall areas (Schradin 2005). In winter-rainfall areas, breeding males show the highest prolactin levels in spring when day length increases and significantly lower prolactin levels in summer when day-length decreases, suggesting a photoperiodic control of prolactin secretion (Schradin 2008). African striped mice from winter-rainfall areas, however, breed in summer after exceptional rainfalls which increase food availability (Schradin, unpublished data). To our knowledge, this offers an unique opportunity to test whether photoperiod or food availability is more important in the regulation of prolactin levels in a free-ranging opportunistically breeding mammal species.

Materials and methods

Study area

The study site was located on Goegap Nature Reserve (41.56°S, 1.60°E) in South Africa and consisted of 20ha. The vegetation type is Succulent Karoo (Cowling *et al.* 1999). The field site was around a dry riverbed and the vegetation was characterized by the evergreen succulent shrub, *Zygophpyllum retrofractum*. Sandy patches get more frequent the larger the distance from the dry riverbed, and especially here annuals (wildflowers and succulents) grow in spring. These plants are especially palatable for striped mice and are a major food source for them in spring (Schradin 2005; Schradin & Pillay 2006).

Trapping and marking of animals

African striped mice were trapped at their nests by using metal live traps similar to Sherman's traps (26×9×9 cm) baited with a mixture of bran flakes and salad oil. Mice were trapped twice every month,, each time for 3 days. Mice were permanently marked using numbered metal ear tags (National Band and Tag Co., Newport, KY, USA). Additionally, each individual was dyed for visual identification with a mark on the pelage (Rapido, Pinetown South Africa). Reproduction was assessed by the presence of pups in the family group of breeding males. Groups were monitored additionally by radio-tracking at least one breeding female per group. During the breeding season, breeding males also carried radio-collars (for details see Schradin et al. 2010).

Blood samples and hormones assays

Sixty blood samples were taken from 48 adult breeding males: 12 samples from 10 males in spring 2009 (8th September-3rd December), 22 samples from 19 males in summer 2009 (3rd March-30th May) and 9 samples from 9 males in summer 2011 (12th February-6th April; both summers, i.e. in 2009 and 2011, with reproduction), and 17 samples from 14 males in summer 2010 (4th February-20th May; summer without reproduction). Among the 48 adult breeding males, we collected a unique blood sample for 37 males; for 7 males, blood samples were taken twice in the same season of the same year; for 3 males, blood samples were taken twice in two different seasons; for one male, blood samples were taken three times: two samples were taken in the same season of the same year and one was taken in another season. To avoid pseudoreplication, we used "male identity" and "year" as random factors in our statistical analysis (see data analyses). Blood samples were collected in the morning (6:00 – 8:00am) when individuals emerged from their nest, controlling for possible circadian rhythms of hormone release. Mice were anaesthetized with di-ethyl ether and a blood sample of 500µl was collected from the sub-lingual vein (Heimann 2006) within less than three minutes, thereby controlling for possible corticosterone effects on prolactin levels (Schradin 2008). After one hour, blood samples were centrifuged two successive times for 10 min. The resulting serum was frozen in aliquots of 60µl for prolactin assays. We used a commercial kit from SPIbio (A05101, rat prolactin) validated by Schradin (2008). The intra- and inter-assay coefficients of variation were 14.57% and 13.38%.

Plant surveys

On the 15th of each month, we did plant surveys in eight plots each of 2 x 2 m within the home ranges of eight groups, using standard protocols (Braun-Blanquet Method; (Werger 1974), recording the number of food plant species in each plot. Monitoring plots were chosen such that the availability of ephemeral and annual plant species was recorded, which are regarded as the high quality food sources for striped mice (Schradin & Pillay 2006). Palatability of food plants was known from direct behavioural observations (Schradin & Pillay 2006).

Data analyses

Statistical analyses were carried out with R 2.15.0 (R Development Core Team 2012). Results are presented as mean ± SEM and significance was accepted at $\alpha \leq 0.05$. We ran two generalized linear mixed effect models (GLMMs) with a Gaussian error distribution and "prolactin levels" as a response variable which was square root transformed to achieve

linearity of residuals. “Male identity” and “year” were added as random effects in the models because 12 blood samples were replicates and our field study covered three successive years. In a first model (GLMM1), we tested whether “photoperiod” (time period when day length is either increasing (i.e. spring) or decreasing (i.e. summer)) and “reproduction” influenced “prolactin levels”. In a second model (GLMM2) we tested whether “photoperiod” and “food availability” influenced “prolactin levels”. We ran two models instead of one because reproduction is highly correlated with food availability (Schradin & Pillay 2006). We used Akaike’s second-order information criteria (AICc) for small sample size to compare the two models. To further test the effect of reproduction on male prolactin levels, we performed pairwise comparisons between “spring”, “summer without reproduction”, and “summer with reproduction” with *post hoc* tests following the Benjamini and Hochberg method (Benjamini & Hochberg 1995).

Results

GLMM1showed a significant effect of reproduction on male prolactin levels ($F_{1,48.5}$ = 5,02; p = 0.03). In GLMM2, “food availability” also influenced significantly male prolactin levels ($F_{1,40.5}$ = 4.03; p = 0.05; Figure 1). “Photoperiod” did not significantly influence male prolactin levels (GLMM1: $F_{1,57}$ = 0.83; p = 0.37; GLMM2: $F_{1,33.4}$ = 0.00; p > 0.99). The AICc of GLMM1 was very slightly lower than the AICc of GLMM2 (172.9034 *vs.* 173.6545). Male prolactin levels during spring were significantly higher than those during summer without reproduction (p = 0.04) but did not differ significantly from prolactin levels during summer with reproduction (Post-Hoc: p = 0.47; Figure 2). Male prolactin levels during summer with reproduction were significantly higher than those during summer without reproduction (p = 0.04).

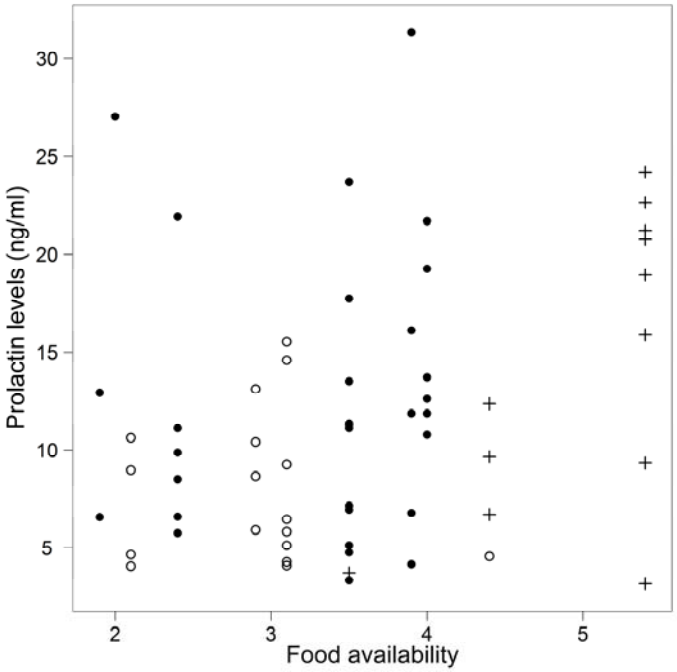

Figure 1. Effect of food availability on male prolactin levels (GLMM1) during spring with reproduction (normal breeding season; +), summer without reproduction (normal dry season; ○), and summer with reproduction (dry season with unexpected rainfall; ●).

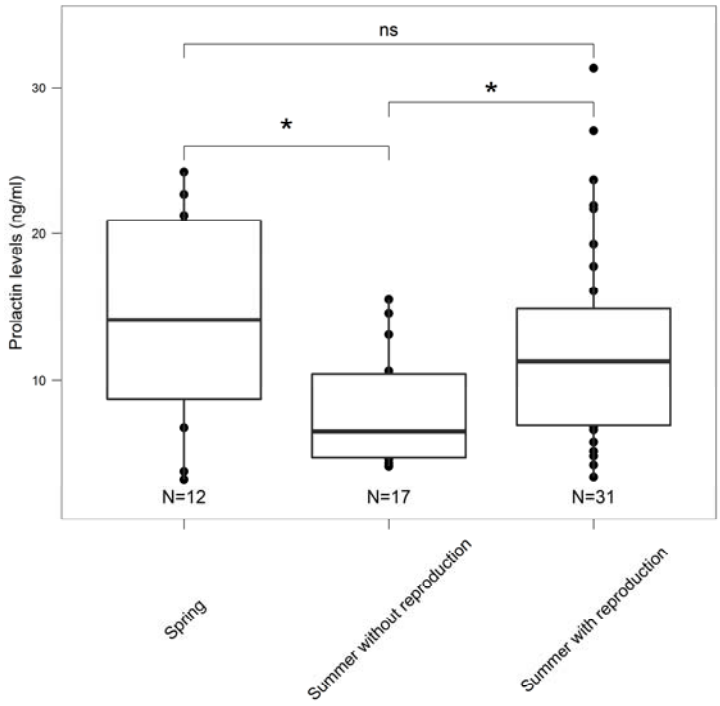

Figure 2. Comparison of male prolactin levels (ng/ml) during spring (normal breeding season; 14.05 ± 2.17 ng/ml), summer without reproduction (normal dry season; 8.01 ± 0.92 ng/ml), and summer with reproduction (dry season with unexpected rainfall; 12.47 ± 1.25 ng/ml). The

median is indicated by the horizontal bar inside the box, the first and third quartile by the box itself, outliers are shown by a black-filled circle. ns: non significant; *****: $p < 0.05$.

Discussion

Up to date, which environmental factors influence prolactin secretion is not clear in opportunistically breeding species, i.e. species coping with unpredictable changes in environmental conditions (Dawson 2008). In the present study we demonstrated that adult breeding males which were reproducing had high prolactin levels independently of whether they reproduced in spring with increasing day length or in summer with decreasing day length. This suggests that other factors that photoperiod must be important in the regulation of prolactin secretion in opportunistically breeding male striped mice. We found a correlation between food availability and prolactin levels that indicates that direct cues related to reproduction might play an important role.

During the natural non-breeding season, male striped mice are non-scrotal (Schradin & Pillay 2005) and do not show spermatogenetic activity (David & Jarvis 1985), but the environmental factors regulating this regression of reproductive activity are not well understood. Neither an experimental prolonged exposure to short day length (winter) nor natural decrease of day length induces spermatogenetic regression in male African striped mice (Jackson & Bernard 1999). Similarly results were found in the pouched mouse, (*Saccostomus campestris*), in which reproduction is not regulated by photoperiod (Bernard & Hall 1995). In the present study, prolactin levels in reproductively active males were highly independent of whether photoperiod was increasing or decreasing, indicating that prolactin secretion might not be photosensitive or that males might have lost sensitivity to photoperiod, which is called photorefractoriness (Nicholls *et al.* 1988). In some seasonal long day breeding species, the breeding season ends while day length is still increasing, and photorefractoriness allows these animals to terminate reproduction before photoperiod starts to decrease. This photorefractoriness has been reported in Japanese quails, *Cortunix japonica* (Robinson & Follett 1982; Guyomarch & Guyomarch 1995), European starlings, *Sturnus vulgaris* (Nicholls et al. 1984; Dawson 1991, 2001), and in Soay sheep, *Ovis aries* (Almeida & Lincoln 1984; Lincoln et al. 2003). While we cannot exclude that photoperiod had an influence on prolactin secretion in male striped mice, in our study, photoperiod could not explain why prolactin levels were higher in summers with than without reproduction and additional factors must be at play in the regulation of prolactin secretion.

Food availability is an important factor limiting reproduction, for instance, in pine siskins, *Spinus pinus* (Watts & Hahn 2012) and passerines (Porlier *et al.* 2012). We found a positive correlation between the number of annual and ephemeral food plant species, which are regarded as high quality food for African striped mice (Schradin 2005; Schradin & Pillay 2006), and prolactin levels, but this correlation was rather weak (Fig. 1) and only approached significance ($p = 0.05$). Similarly, food supply during the winter non-breeding season increased testis and epididymis size as well as spermatogenesis activity in male striped mice (Jackson & Bernard 2005). Together, these studies indicate that food availability might be one of several factors influencing prolactin secretion and reproduction. Food availability is typically a function of previous rainfall (Schradin & Pillay 2006). Water availability, humidity, and barometric pressure may be other factors influencing prolactin secretion, as reported in California mice, *Peromyscus californicus* (Nelson et al. 1995) and Darwin's ground finches, *Geospiza fuliginosa* (Hau *et al.* 2004). Rainfall reliably predicts a future increase of food availability for African striped mice but whether prolactin levels increase immediately after rainfall or only after an increase of food availability remains unknown.

Interestingly, prolactin levels can also differ between males of different reproductive tactics in house finches (Badyaev & Vleck 2007) and in African striped mice (Schradin 2008). Males show higher prolactin levels when they switch from a solitary non-paternal to a group-living paternal reproductive tactic during the breeding season (Schradin & Yuen 2011). This physiological flexibility of male African striped mice suggests that prolactin has more important effects on reproductive behaviours (including paternal care) than it does on timing of reproduction per se. Thus, the regulation of prolactin levels appears to depend on a complex interaction between environmental factors timing reproduction (e.g. food availability) and ecological factors mediating social flexibility (i.e. population density and reproductive competition) (Schradin et al. 2010; Schoepf & Schradin 2012; Schradin et al. 2012).

Our study is a step forward to understanding the regulation of prolactin release in opportunistically breeding species. Experimental studies will be essential to demonstrate which non-photoperiodic cues are at play. As higher prolactin levels are only reported during breeding events, prolactin influenced likely gonadal activity and paternal care (Schradin 2008). In harsh habitats, prolactin secretion might also respond to energetic constraints due to parenting effort as in bird species (Angelier & Chastel 2009). These hypotheses are not mutually exclusive and testing them will help to go toward a full understanding of prolactin regulation and its function in species coping with unpredictable environmental changes.

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References

Almeida, O. F. X. & Lincoln, G. A. 1984. Reproductive photorefractoriness in rams and accompanying changes in the patterns of melatonin and prolactin secretion. *Biology of Reproduction*, **30**, 143-158.

Angelier, F. & Chastel, O. 2009. Stress, prolactin and parental investment in birds: A review. *General and Comparative Endocrinology*, **163**, 142-148.

Badyaev, A. V. & Vleck, C. M. 2007. Context-dependent development of sexual ornamentation: implications for a trade-off between current and future breeding efforts. *Journal of Evolutionary Biology*, **20**, 1277-1287.

Bartke, A. 2004. Prolactin in the male: 25 years later. *Journal of Andrology*, **25**, 661-666.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological*, **57**, 289-300.

Bernard, R. T. F. & Hall, J. 1995. Failure of the estrous-cycle and spermatogenesis to respond to day length in a subtropical african rodent, the pouched mouse (saccostomus-campestris). *Biology of Reproduction*, **52**, 1291-1295.

Buntin, J. D. 1996. Neural and hormonal controls of parental behavior in birds. *advances in the study of behavior*, **25**, 161-213.

Christensen, D. & Vleck, C. M. 2008. Prolactin release and response to vasoactive intestinal peptide in an opportunistic breeder, the zebra finch (Taeniopygia guttata). *General and Comparative Endocrinology*, **157**, 91-98.

Cowling, R. M., Esler, J. J. & Rundel, P. W. 1999. Namaqualand, South Africa – an overview of a unique winter-rainfall desert ecosystem. *Plant Ecology*, **142**, 3-21.

Curlewis, J. D. 1992. Seasonal prolactin secretion and its role in seasonal reproduction - a review. *Reproduction Fertility and Development*, **4**, 1-23.

David, J. H. M. & Jarvis, J. U. M. 1985. Population fluctuations, reproduction and survival in the striped fieldmouse rhabdomys-pumilio on the cape flats, south-africa. *Journal of Zoology*, **207**, 251-276.

Dawson, A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 1621-1633.

Dawson, A. 2001. The effects of a single long photoperiod on induction and dissipation of reproductive photorefractoriness in European starlings. *General and Comparative Endocrinology*, **121**, 316-324.

Dawson, A. 1991. The induction of photorefractoriness and molt in starlings, sturnus-vulgaris, by continuous or intermittent long days. *Physiological Zoology*, **64**, 1252-1261.

Goldman, B. D., Song, C. K. & Bartness, T. J. 2008. Seasonal Rhythms: Seasonal hormonal changes and behavior. In: *Encyclopedia of Neuroscience* (Ed. by L. R. Squire). Oxford: Academic Press.

Guyomarch, C. & Guyomarch, J. C. 1995. Molting cycles in european quail (coturnix-coturnix coturnix) under constant photoperiodic conditions. *Biological Rhythm Research*, **26**, 292-305.

Hall, T. R., Harvey, S. & Chadwick, A. 1986. Control of prolactin secretion in birds - a review. *General and Comparative Endocrinology*, **62**, 171-184.

Hau, M., Wikelski, M., Gwinner, H. & Gwinner, E. 2004. Timing of reproduction in a Darwin's finch: temporal opportunism under spatial constraints. *Oikos*, **106**, 489-500.

Heimann, M. 2006. Development and validation of the method of sublingual blood sampling in mice and other small rodents, University of Zurich.

Jackson, C. & Bernard, R. T. F. 2005. Effects of supplementary food on the winter inhibition of reproduction in male and female four-striped field mice (Rhabdomys pumilio). *Reproduction Fertility and Development*, **17**, 393-400.

Jackson, C. & Bernard, R. T. F. 1999. Short day length alone does not inhibit spermatogenesis in the seasonally breeding four-striped field mouse (Rhabdomys pumilio). *Biology of Reproduction*, **60**, 1320-1323.

Johnston, J. D. 2004. Photoperiodic regulation of prolactin secretion: changes in intra-pituitary signalling and lactotroph heterogeneity. *Journal of Endocrinology*, **180**, 351-356.

Lincoln, G. A., Andersson, H. & Clarke, I. J. 2003. Prolactin cycles in sheep under constant photoperiod: Evidence that photorefractoriness develops within the pituitary gland independently of the prolactin output signal. *Biology of Reproduction*, **69**, 1416-1423.

Lincoln, G. A., McNeilly, A. S. & Cameron, C. L. 1978. Effects of a sudden decrease or increase in daylength on prolactin secretion in ram. *Journal of Reproduction and Fertility*, **52**, 305-311.

Nelson, R. J. 2005. *An Introduction to BEHAVIORAL ENDOCRINOLOGY*, Third edn. Sunderland: Sinauer Associates, INC.

Nelson, R. J., Gubernick, D. J. & Blom, J. M. C. 1995. Influence of photoperiod, green food, and water availability on reproduction in male california mice (peromyscus-californicus). *Physiology & Behavior*, **57**, 1175-1180.

Nicholls, T. J., Goldsmith, A. R. & Dawson, A. 1988. Photorefractoriness in birds and comparison with mammals. *Physiological Reviews*, **68**, 133-176.

Nicholls, T. J., Goldsmith, A. R. & Dawson, A. 1984. Photorefractoriness in european starlings - associated hypothalamic changes and the involvement of thyroid-hormones and prolactin. *Journal of Experimental Zoology*, **232**, 567-572.

Paul, M. J., Zucker, I. & Schwartz, W. J. 2008. Tracking the seasons: the internal calendars of vertebrates. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 341-361.

Porlier, M., Charmantier, A., Bourgault, P., Perret, P., Blondel, J. & Garant, D. 2012. Variation in phenotypic plasticity and selection patterns in blue tit breeding time: between- and within-population comparisons. *Journal of Animal Ecology*, **81**, 1041-1051.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Robinson, J. E. & Follett, B. K. 1982. Photoperiodism in japanese quail - the termination of seasonal breeding by photorefractoriness. *Proceedings of the Royal Society B-Biological Sciences*, **215**, 95-116.

Schoepf, I. & Schradin, C. 2012. Better off alone! Reproductive competition and ecological constraints determine sociality in the African striped mouse (Rhabdomys pumilio). *Journal of Animal Ecology*, **81**, 649-656.

Schradin, C. 2008. Differences in prolactin levels between three alternative male reproductive tactics in striped mice (Rhabdomys pumilio). *Proceedings of the Royal Society B-Biological Sciences*, **275**, 1047-1052.

Schradin, C. 2005. When to live alone and when to live in groups : ecological determinants of sociality in the African striped mouse (Rhabdomys pumilio, Sparrman, 1784). *Belgian Journal of Zoology*, **135**, 77-82.

Schradin, C. & Yuen, C.-H. 2011. Hormone levels of male African striped mice change as they switch between alternative reproductive tactics. *Hormones and Behavior*, **60**, 676-680.

Schradin, C. & Pillay, N. 2006. Female striped mice (Rhabdomys pumilio) change their home ranges in response to seasonal variation in food availability. *Behavioral Ecology*, **17**, 452-458.

Schradin, C. & Anzenberger, G. 1999. Prolactin, the hormone of paternity. *News in Physiological Sciences*, **14**, 223-231.

Schradin, C., König, B. & Pillay, N. 2010. Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515-521.

Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., König, B. & Pillay, N. 2012. Social flexibility and social evolution in mammals: a case study of the African striped mouse (Rhabdomys pumilio). *Molecular Ecology*, **21**, 541-553.

Sharp, P. J. 2005. Photoperiodic regulation of seasonal breeding in birds. In: *Trends in Comparative Endocrinology and Neurobiology* (Ed. by H. Vaudry, E. Roubos, L. Schoofs, G. Fiik & D. Larhammar), pp. 189-199.

Sharp, P. J., Dawson, A. & Lea, R. W. 1998. Control of luteinizing hormone and prolactin secretion in birds. *Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology*, **119**, 275-282.

Smale, L., Nelson, R. J. & Zucker, I. 1988. Daylength influences pelage and plasma prolactin concentrations but not reproduction in the prairie vole, microtus-ochrogaster. *Journal of Reproduction and Fertility*, **83**, 99-106.

Steger, R. W., Bartke, A., Goldman, B. D., Soares, M. J. & Talamantes, F. 1983. Effects of short photoperiod on the ability of golden hamster pituitaries to secrete prolactin and gonadotropins in vitro. *Biology of Reproduction*, **29**, 872-878.

Watts, H. E. & Hahn, T. P. 2012. Non-photoperiodic regulation of reproductive physiology in the flexibly breeding pine siskin (Spinus pinus). *General and Comparative Endocrinology*, **178**, 259-264.

Werger, M. J. A. 1974. On concept and techniques applied in the Zürich-Montpellier method of vegetation survey. *Bothalia*, **11**, 309-323.

Wingfield, J. C. 2008. Organization of vertebrate annual cycles: implications for control mechanisms. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 425-441.

General Discussion

General Discussion

The aim of the present PhD project was to study within an evolutionary framework the proximate mechanisms of behavioural and physiological flexibility, that is the reversible phenotypic changes in physiology and behaviour during the lifespan of an organism (Piersma & Drent 2003). For this, I investigated the endocrine mechanisms that allow individuals to quickly adapt to environmental changes, optimizing their reproductive success. I used alternative reproductive tactics (ARTs) as a suitable framework to address the role of hormones in physiological, morphological, and behavioural flexibility (Taborsky et al. 2008). So far, many studies on endocrine mechanisms of ARTs were correlative and few experimental studies addressed the role of hormones in the regulation of ARTs (Oliveira et al. 2008). In the present PhD thesis, I experimentally studied the role of testosterone in ARTs in a mammal species: the African striped mouse (*Rhabdomys pumilio*). Specifically, I focused on the switch of juvenile male African striped mice from group-living helpers to solitary-living roamers. Secondly, I demonstrated flexibility in secretion of prolactin, a hormone that is thought to play an important role in the regulation of ARTs in African striped mice (Schradin 2008b; Schradin & Yuen 2011).

Endocrine mechanisms of ARTs

The Relative Plasticity Hypothesis

The relative plasticity hypothesis (RPH) relies on two main types of influences of steroid hormones (Moore 1991): organizational and activational effects (Phoenix et al. 1959). The RPH predicts that organisational effects of steroid hormones determine, at early life stages, the reproductive tactics of individuals for the rest of their life (i.e. fixed ARTs) and activational effects of steroid hormones allow individuals to switch from one ART in another one throughout life (i.e. plastic ARTs; see the two first RPH predictions of the endocrine mechanisms regulating fixed and plastic ARTs in Table 1). The second generation of the RPH shows new refinements in the predicted endocrine mechanisms regulating ARTs (Moore et al. 1998). The organisational effects of steroid hormones would still cause the development in a given ART leading to a permanent phenotype for species showing fixed ARTs (see Table 1). The refinements of the predicted endocrine mechanisms concerned species showing plastic ARTs. Moore et al (1998) predicted that the effects of steroid hormones on the regulation of ARTs would be reversible or irreversible, i.e. sequential (see Table 1). For instance, in tree lizards, *Urosaurus ornatus*, males show both fixed and plastic ARTs: males with orange dewlaps can follow either a nomadic tactic or sedentary tactic in a reversible manner

depending on the rainfall abundance (reversible ARTs), whereas males with blue dewlaps remain territorial regardless of the environmental conditions (fixed ARTs) (Knapp et al. 2003). When environmental condition are harsher (i.e. drier) both sedentary males (males with orange dewlaps) and territorial males (males with blue dewlaps) showed an increase of corticosterone levels, but only sedentary males showed a decrease of testosterone levels and become nomadic males (Knapp et al. 2003). It has been demonstrated that an experimental increase of corticosterone decrease testosterone levels in males with orange dewlaps but not in males with blue dewlap. Thus, in tree lizards, the corticosterone effect on testosterone levels might be the endocrine mechanism regulating the reversible switch that allows males with orange dewlaps to follow either nomadic or sedentary tactics, whereas, only organizational testosterone effects determine the territorial tactic of male with blue dewlap (Hews et al. 1994; Hews & Moore 1996). In marine iguanas, *Amblyrhynchus cristatus,* the reversibility of ARTs has been experimentally demonstrated through a testosterone mechanism: an experimental decrease of testosterone in territorial males caused them to lose their territory and an increase of testosterone in satellite males caused them to establish a territory (Wikelski et al. 2005). In sequential ARTs, individuals can switch from one ART in another one but only in a permanent order. This irreversibility has important consequences on the endocrine mechanisms that would be at play: 1) as the sequential changes in ARTs are permanent, the effect of steroid hormones would be more organizational like during adulthood (Table1); 2) as steroid hormones would only operate during the changes in ARTs (organizational and activational effects should occur in one step), the differences in hormone levels between males of different ARTs should only appear during the switch (Table 1) (Moore et al. 1998).

Testing the relative plasticity hypothesis in African striped mice

Schradin et al (Schradin 2008b; Schradin et al. 2009b; Schradin & Yuen 2011) tested the first prediction of the RPH in male African striped mice (Table 1) – steroid hormone level differences of species showing plastic ARTs should be visible at later life stages (Moore 1991). In African striped mice, adult males of different ARTs differ in prolactin, testosterone, and corticosterone levels (Schradin 2008b; Schradin et al. 2009b), and individuals that change their tactics change their hormonal profiles after the tactic switch (Schradin & Yuen 2011). These studies confirmed the first prediction of the RPH – male African striped mice of different ARTs differ in hormone levels (Schradin 2008b; Schradin et al. 2009b; Schradin & Yuen 2011). Importantly, the authors also stressed the need to demonstrate that individuals

change their hormone profiles when they change tactics (Schradin & Yuen 2011), because inter-individual hormone level differences between ARTs do not necessarily imply intra-individual hormone levels differences between ARTs (Eikenaar et al. 2011).

Table 1. Endocrine predictions from the first and second generation of the relative plasticity hypothesis (RPH) for ARTs (from Moore et al. 1998; Oliveira et al. 2008).

RPH generations	**ART types**	**1st endocrine prediction**: Individuals of different ARTs show hormone level differences.	**2nd endocrine prediction**: Hormone level differences cause the development of different ARTs.
1st generation of the RPH (Moore 1991)	**Fixed ARTs**	Hormone level differences visible at early life stages only.	Hormones operate at early life stages only (organizational effects).
	Plastic ARTs	Hormone level differences visible at later life stages only.	Hormones operate at later life stages only (activational effects).
2nd generation of the RPH (Moore et al. 1998)	**Fixed ARTs**	Hormone level differences visible at early life stages only.	Hormones operate at early life stages only (organizational effects).
	Reversible ARTs	Hormone level differences visible at later life stages only.	Hormones operate at later life stages only (activational effects).
	Sequential ARTs	Hormone level differences visible during the tactic switch only.	Hormones operate in a sequential order (organisational + activational effects) throughout lifespan.

In the present PhD thesis, I tested the second prediction of the RPH (Table 1), which states that changes in hormonal levels cause reproductive tactic switch (Moore 1991). However, an experimental increase of testosterone levels in free-ranging juvenile male group-living juvenile helpers did not cause the development into the roaming tactic (chapter 3). How can I explain the failure to demonstrate the second prediction of the RPH (chapter 3) that contrasts with the success of previous studies demonstrating the first prediction of the RPH (Schradin et al. 2009b; Schradin & Yuen 2011)? One may argue that the juvenile male group-living helpers used in my field experiment (chapter 3) were too young to develop into solitary-living roamers. However, these juvenile male group-living helpers reached the age of puberty, i.e. 4 weeks old (chapter 3), and a previous experimental study showed that such juvenile males can disperse and become solitary-living roamers (Schoepf & Schradin 2012a). One may also argue that the testosterone treatment of 14 days (chapter 3) was too short to allow juvenile male group-living helpers to disperse and to become solitary-living roamers. However, juvenile male group-living helpers typically increase their home range for only

about 1 week before becoming solitary-living roamers (Schradin, unpublished data). In other words, testosterone-treated juvenile male group-living helpers had twice much time to disperse in my experiment (chapter 3). I rather suggest that the environmental and especially social conditions during this experiment (chapter 3) did not favour dispersal of testosterone-treated males.

The breeding season during this experiment was unexpectedly dry and only 42 % of adult females did reproduce (chapter 3). There were also more group-living living females than solitary-living females in the studied population (the ratio of solitary- to group-living females was 0.36; chapter 3) while it had been predicted that juvenile group-living males disperse when this ratio is above 1 (Schradin & Lindholm 2011). In sum, the fact that testosterone-treated males did not disperse is most likely due to the decision to disperse relying on environmental signals (Schoepf & Schradin 2012a; Schradin et al. 2012b) and not on testosterone signals alone (chapter 3). While I could not confirm the second prediction of the RPH in African striped mice (chapter 3), my results support the idea of a diversity of mechanisms regulating ARTs (Moore et al. 1998). In future studies, I encourage to experimentally test the hypothesis that either food availability-related signals or mating partner-related ones play a role in the decision to disperse.

Role of testosterone in physiological and behavioural changes

The experimental increase of testosterone in juvenile male group-living helpers demonstrated that testosterone plays an important regulatory role in physiological, morphological, and behavioural differences between juvenile male group-living helpers and solitary-living males (Table 2). These physiological, morphological, and behavioural changes indicate that these traits are plastic through a testosterone-related mechanism (chapter 2; 3). In other words, while exogenous testosterone did not cause males to become solitary-living roamers, my results demonstrated that an increase of testosterone levels in the blood stream caused physiological, morphological, and behavioural changes related to the development of the roaming tactic.

Sexual maturation is typically delayed in male group-living African striped mice (Schradin et al. 2009a) even though philopatric helpers produce as much testosterone in the testes as solitary males, but secrete less testosterone (Schradin et al. 2012a). Exogenous testosterone quickly enhanced sexual maturation (within 14 days) (chapter3). Thus, my results support the idea that the release of testosterone from the testes in philopatric helpers allow them to quickly become reproductively active (increase of spermatogenesis activity and testis

development) when they decide to disperse and become either solitary-living roamers or dominant territorial breeders (Schradin et al. 2012a).

Table 2. Physiological, morphological, and behavioural changes caused by exogenous testosterone in juvenile male group-living helpers. + indicates a significant effect of testosterone on the trait; - indicates no significant effect of testosterone on the trait.

Physiological changes	Onset of spermatogenesis +
	Decrease of corticosterone levels +
Morphological changes	Becoming scrotal (testes fully descended) +
	Increase of testes size +
	Increase of epididymis size +
Behavioural changes	Increase of homes ranges size +
	Decrease of anxiety +
	Increase of boldness +
	Decrease of alloparental care –
	Increase of aggressiveness –
	Becoming solitary –

The high corticosterone levels observed in philopatric helpers is the potential mechanism that inhibits the testosterone release in the bloodstream (Schradin et al. 2009a). Exogenous testosterone quickly reduced corticosterone secretion: after only one day of testosterone treatment corticosterone levels of testosterone-treated males were already significantly lower than of control males (chapter 2). This result has two implications: 1) the decrease of corticosterone due to exogenous testosterone may lift the inhibition of the testosterone secretion from the testes; 2) the decrease of anxiety induced by exogenous testosterone might be due to the decrease of the basal corticosterone levels which then could explain why they had larger home ranges. In other words, the lower basal corticosterone may have decreased the stress reactivity (corticosterone secretion) when testosterone-treated males faced stressing situations (chapter 2) while increasing their home ranges (chapter 3).

I suggested an idea that was initially formulated by Holekamp et al (1984), which is that testosterone may not directly cause dispersal but rather facilitate it through an increase of boldness and a decrease of anxiety (Aikey et al. 2002). With my experimental testosterone manipulations conducted in field and captive conditions, I support this idea: in field condition, an increase of testosterone levels caused juvenile male group-living helpers to expand their home ranges (chapter 3) and in captive condition, an increase of testosterone increased boldness and decreased anxiety in philopatric helpers (chapter 2).

When juvenile male group-living helpers were kept in their family unit in captive condition, an experimental increase of testosterone did not decrease alloparental care and aggressive behaviour (chapter 2) as observed in field condition in dispersing mice (Schoepf & Schradin 2012a). This phenomenon has been called behavioural insensitivity to testosterone (Lynn 2008). Insensitivity to experimentally increased testosterone levels has been reported for parental care and aggressive behaviour in chestnut-collared longspurs, *Calcarius ornatus* (Lynn et al. 2002; Lynn & Wingfield 2008), and for courtship behaviour in ring doves, *Streptopelia risoria* (Fusani & Hutchison 2003), suggesting that the brain was not ready to respond to the testosterone signals in specific social and environmental contexts (Gleason et al. 2009). There is evidence that different environmental conditions correlate with differences in brain sensitivity to testosterone (Canoine et al. 2003). For instance, in pied flycatchers (*Ficedula hypoleuca*) aromatase activity is higher in males during territory establishment than during egg laying (Silverin et al. 2004). In African striped mice, changes in environmental and social conditions (e.g. food availability and receptive female availability) may prepare the brain of juvenile male group-living helpers to respond, in terms of alloparental care and aggressiveness, appropriately to testosterone signals by increasing the sensitivity of specific neural pathways to testosterone and testosterone metabolites, i.e. by increasing steroid receptor density and by increasing aromatase activity (Canoine et al. 2003; Silverin et al. 2004; Lynn 2008). Thus, we might need to consider that the changes in male reproductive traits during the tactic switch is mediated by several testosterone mechanisms that are independent from each other (Finch & Rose 1995; Sinervo & Svensson 1998). This could explain why there are responses to testosterone in non-social behaviour (i.e. boldness, activity, anxiety-like behaviour) but not in social behaviour (i.e. alloparental care and aggressive behaviour).

Another simpler explanation might be that the insensitivity of alloparental care to testosterone is because testosterone plays no role in the regulation of alloparental care. In my study about the factors that influence alloparental care (chapter 1), I showed that testosterone levels in philopatric helpers did not correlate with alloparental care (time spent in the nest with pups, huddling the pups, and licking the pups). In another study done in captivity, singly housed males had much higher testosterone levels than their family housed alloparental brothers, but there was no difference in their response towards pups presented to them (Schradin et al. 2013).

I studied the factors influencing alloparental care because the mechanism regulating alloparental care might also be involved in the tactic switch from philopatric helper to

solitary-living roamer. It is generally accepted that the dispersing sex provide less alloparental care than the non-dispersing sex in bird and mammal species (Cockburn 1998; Clutton-Brock et al. 2002; Johnstone & Cant 2008), and in striped mice dispersal is male-biased (Solmsen et al. 2011). Accordingly I found that male helpers showed more alloparental care than female helpers (chapter 1). Corticosterone levels were negatively correlated with alloparental care in female helpers but not in male helpers (chapter 1). Different mechanisms may operate between male and female helpers in regulating alloparental care, as described in prairie voles, *Microtus ochrogaster* (Roberts et al. 1996; Roberts et al. 1998). I found no evidence that testosterone and corticosterone play a role in the expression of male alloparental care (chapter 1; 2). In terms of mechanism(s) regulating tactic switch, changes in alloparental care expression might be a consequence of the tactic switch – dispersing African striped mice with high testosterone and low corticosterone levels were more aggressive towards pups than before these same males dispersed (Schoepf & Schradin 2012b) – which is not induced by up-regulation of testosterone levels.

A hypothetical model of the tactic switch from philopatric helper to solitary-living roamer

My integrative approach is to consider the relationships between environmental cues and hormonal signals in the regulation of ARTs. Population density and reproductive competition regulate the social system of African striped mice (Schradin et al. 2010a; Schoepf & Schradin 2012a). On the individual level, this means that both males and females can change their reproductive tactics when these two factors change (Schradin et al. 2012b). Integrating predictions about environmental influences on the tactic switch with the 2nd prediction of the RPH, i.e. changes in hormone levels cause tactic switch (Moore 1991; Moore et al. 1998), might be the key to understand the mechanism at play in my case of study, i.e. the tactic switch between philopatric helpers and solitary-living roamers, and certainly in more species showing ARTs (Figure 1). Thus, the relationships between hormones (e.g. testosterone) and environmental factors have to be studied in more details, specifically, in two main topics:

1) Which and how environmental cues regulate the release of testosterone from the testis in the bloodstream and whether the testosterone release causes physiological, morphological, and behavioural changes as observed in chapter 2 and 3.
2) Which environmental cues and which neural pathways are involved in tactic switch (i.e. neural pathways involved in the decision to disperse) and whether these neural pathways are modulated by testosterone (i.e. whether an increase of testosterone levels

increase the probability to disperse in terms of environmental conditions).

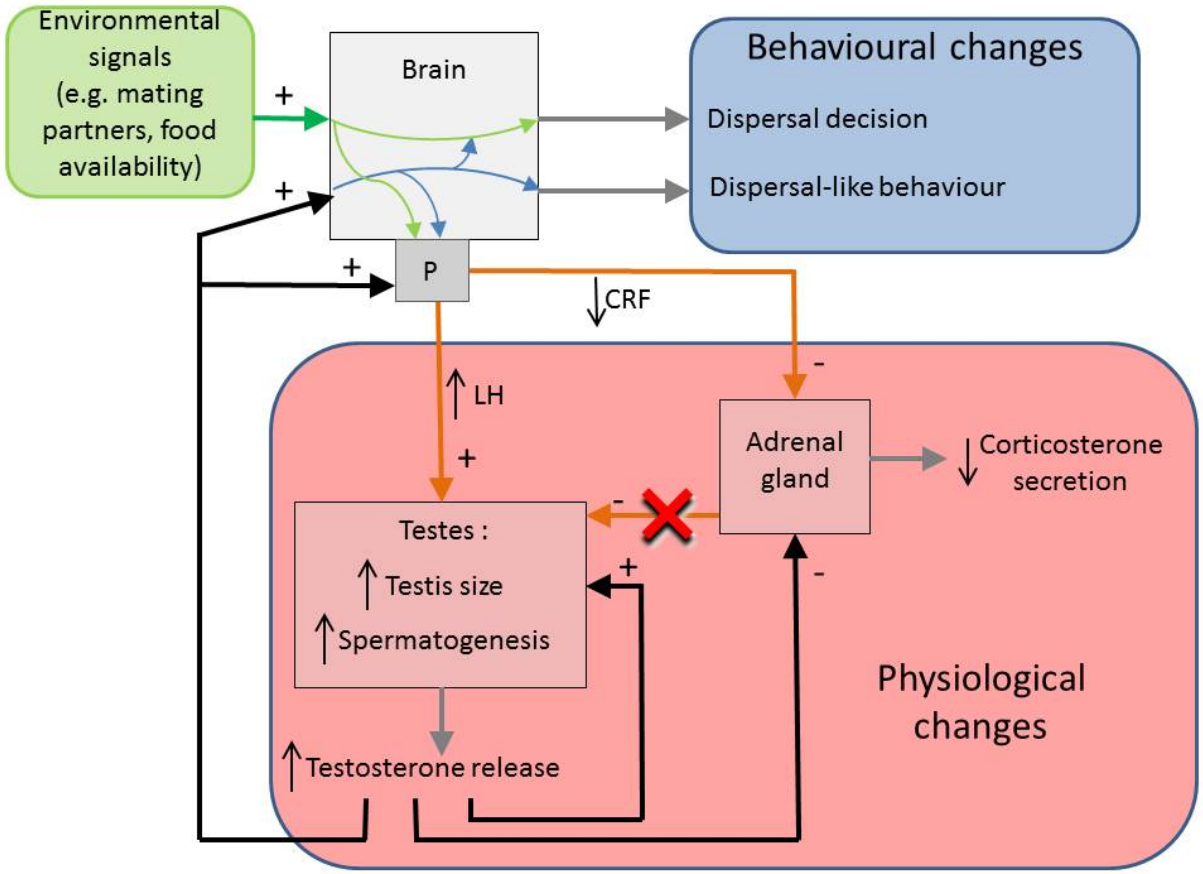

Figure 1. Hypothetical model of the male tactic switch from juvenile group-living helper to solitary-living roamer. The model integrates environmental factors with the testosterone-related mechanisms in physiological (red box; for simplicity morphological changes are included in physiological changes) and behavioural changes (blue box; including the dispersal decision). Green arrow: environmental influences; black arrows: testosterone (or testosterone metabolites) influences; orange arrows: other hormone influences (i.e. luteinizing hormones (LH), corticotropin-releasing factors (CRF), corticosterone); grey arrows: physiological and behavioural outcomes from the hormone and environmental influences; arrows inside the brain: green ones: neural pathways influenced by environmental factors; blue ones: neural pathways influenced by testosterone (or testosterone metabolites); + indicates positive effects; - indicates negative effects; X indicates the lift of corticosterone inhibition on testes function.

Testosterone release, an important step in ART development

During the dispersal phase, the factors inhibiting the testosterone release in the blood stream should not operate, allowing blood testosterone levels to increase quickly. Thus, testosterone

can quickly reach target tissues allowing the mice to undergo the behavioural, physiological and morphological changes necessary for dispersal (chapter 3). The release of the "testosterone stock" from the testes appears to be a critical event during the tactic switch. Which are the potential factors that could trigger the release of testosterone from the testes? The ability of corticosterone to directly alter gonadal function is generally accepted (Rivier & Rivest 1991). The high corticosterone levels observed in philopatric helpers is a potential mechanism that inhibits the testosterone release in the bloodstream (Schradin et al. 2009a; Schradin et al. 2012a). Even though I demonstrated that testosterone decreased corticosterone levels (chapter 2; 3), I was not able to differentiate between endogenous testosterone (testosterone secretion from the testes) and exogenous testosterone secreted by the implants, which is why it was impossible to demonstrate that testosterone from the testes was released during my experiments (chapter 2; 3). An experimental decrease of corticosterone levels using an inhibitor of corticosterone synthesis (methyparone) in male helpers could test the role of corticosterone in testosterone release. A second potential candidate is proopiomelanocortin (POMC). POMCs are precursors of adrenocorticotropin (ACTH) – an important neurohormone regulating corticosterone levels. POMC can directly regulate gonadal function (Gerendai et al. 1984; Boitani et al. 1985) and POMC mRNA expression in the testes can be regulated by environmental factors (i.e. temperature) (Endo & Park 2004) suggesting that POMC itself may mediate the influence of environmental conditions on testis activity (steroidogenesis) (Endo & Park 2004). Another candidate may also be the luteinising hormone (LH). This hormone regulates the production and secretion of testosterone in the testes. Although LH has never been measured in African striped mice, LH secretion should be partially inhibited in male helpers as the latter produces as much testosterone in the testes as solitary-living roamers, but secrete less testosterone than solitary-living roamers (Schradin et al. 2012a). LH secretion can be directly influenced by environmental stimuli such as female odours (Johnston & Bronson 1982), seasonal changes (Pelletier et al. 1982), and food availability (Cameron & Nosbisch 1991). Thus, increase of LH secretion, due to environmental changes which cause dispersal, may enhance the secretion of testosterone from the testes. It would be interesting to measure the LH levels before, during, and after the dispersal phase, with the prediction that LH levels in the blood stream increase through these different stages. Finally, leptin is also known to inhibit testosterone secretion at the testis levels in rat (Tena-Sempere et al. 1999). Interestingly, in African striped mice, male philopatric helpers show the highest leptin levels (Schradin, unpublished data). To investigate the role of leptin in testosterone secretion, a first step would be to test for a negative

correlation between leptin and testosterone levels in male philopatric helpers.

Environmental activation of neural pathways modulated by testosterone

Before juvenile male group-living helpers become solitary-living roamers, they undergo sexual maturation (Schradin et al. 2009a; Schoepf & Schradin 2012a) and they then should be able to show sexual arousal when encountering adult females, as in many other taxa (Bancroft 2005; Ball & Balthazart 2011; Forlano & Bass 2011; Wade 2011). Sexual arousal is regulated at the level of the mesolimbic dopamine pathway and the limbic systems such as the medial preoptic area (MPOA), the bed nucleus of the stria terminalis (BNST), and the medial amygdala (MeA) in both mammals and birds (Hull 1995; Hull et al. 1999; Dominguez & Hull 2005; Hull & Dominguez 2007; Kleitz-Nelson et al. 2010a; Kleitz-Nelson et al. 2010b). These brain areas show high concentration of androgen receptors (Simerly 1995; Yahr 1995) and receive many afferent projections from the main and accessory olfactory bulb (Wood & Newman 1995). This is important because olfaction is the most important sensory modality in rodent reproduction (Brennan & Keverne 2004; Keverne 2004; Brennan & Kendrick 2006; Brennan & Zufall 2006), and olfactory sexual cues (i.e. pheromones) are well-known to activate neurons in limbic circuits (Meredith 1998). In African striped mice, males discriminate female odour of different reproductive status and prefer the odours of oestrus females (Bennett & Pillay 2001), suggesting a role of female odours in the expression of male reproductive behaviour. Both hormonal signals and environmental cues can be integrated in the limbic system to stimulate sexual motivation (Wood & Coolen 1997; Been & Petrulis 2011). For instance, stimulation of medial amygdala (MeA) by testosterone in the absence of ipsilateral chemosensory signals fails to stimulate sexual motivation in male Syrian hamsters, *Mesocricetus auratus* (Wood & Coolen 1997). Furthermore, oestrus female odours induce dopamine release in the POA, the BNST (Hull 1995; Dominguez & Hull 2005), and the accumbens nucleus (Balfour et al. 2004) enhancing in turn sexual motivation. Not only in mammals but also in amphibians, social cues (mating calls) positively feedback the HPG axis, specifically activating the gonadotropin-releasing hormone (GnRH) neurons that increase GnRH and by this androgen levels (Wilczynski et al. 1993; Burmeister & Wilczynski 2005; Wilczynski et al. 2005). A similar mechanism that involves a synergetic action of hormonal signals (testosterone) and environmental cues (mates-related cues) may cause philopatric males to leave the group and start searching for mating partner (sexual arousal). One may test this hypothesis with the prediction that the absence of testosterone signal (e.g. via gonadectomy), or the absence of environmental cues (e.g. mate odours), and the absence of

both factors prevent dispersal in juvenile male group-living helpers.

Environment and flexibility in prolactin secretion

From my experimental testosterone manipulation studies, the importance of environmental factors in the regulation of the physiological and behavioural flexibility in the African striped mouse became a valid hypothesis. Male striped mice of different ARTs show differences in steroid and prolactin hormone levels during the breeding season in spring (Schradin 2008b; Schradin et al. 2009b), but not during the non-breeding season in summer (Schradin 2008b, a). Many other species show seasonal hormonal variation associated with reproductive activity (Karsch et al. 1984), which is often regulated by seasonal changes in photoperiod (Sharp 2005). For these species, changes in behaviour, physiology, and morphology take place during a so-called "physiological window", within which breeding can occur and which is limited by photoperiod (Dawson 2008). However, I demonstrated that photoperiod did not correlate with the prolactin variation of dominant territorial breeding males (chapter 5). In summers (the typical non-breeding) with high food availability, dominant territorial breeding males had significantly higher prolactin levels than in summers with low food availability, indicating that prolactin secretion is flexibly regulated by either food availability directly or other environmental factors correlated with food availability. Food availability in our study area is a function of previous rainfall (Schradin & Pillay 2006). Thus, rainfall might be a reliable cue for a future increase of food availability for African striped mice. Other factors related to rainfall such as water availability, humidity, and barometric pressure could also be at play in prolactin regulation (Nelson et al. 1995; Hau et al. 2004). However, whether prolactin levels increase immediately after rainfall or only after an increase of food availability remains to be studied.

My results and other previous studies on striped mice (Jackson & Bernard 2005) indicate that territorial breeding males are ready to undergo quick physiological and behavioural changes as soon as the environmental conditions are sufficient for breeding. Thus, it is tempting to conclude that flexibility in prolactin secretion is adaptive in male African striped mice. For this, one would need to demonstrate that up-regulation of prolactin ensures higher individual reproductive success. Interestingly, prolactin levels also differ between males of different reproductive tactics in African striped mice (Schradin 2008b). Male African striped mice show higher prolactin levels after they switched from a solitary-living non-paternal tactic (i.e. solitary-living roamers) to a group-living paternal reproductive tactic (i.e. territorial breeding males) during the breeding season (i.e. spring) (Schradin &

Yuen 2011). The differences in prolactin levels between and within males of different ARTs (Schradin 2008b; Schradin & Yuen 2011) suggests that flexibility in prolactin secretion has more important effects on reproductive behaviours (e.g. paternal care) than it does on timing reproduction per se. Under conditions of high population density, territorial breeding males have higher reproductive success than males being philopatric helpers or solitary-living roamers, but not under intermediate population density (Schradin & Lindholm 2011). The regulation of prolactin levels may depend on a complex interaction between environmental factors timing reproduction (food availability) (chapter 4) and the chosen reproductive tactic depending on factors mediating social flexibility (population density) (Schradin et al. 2010b; Schoepf & Schradin 2012a; Schradin et al. 2012c). The ability of male African striped mice to choose among three ARTs, where up- or down- regulation of prolactin levels takes place depending on environmental factors in a way that males may optimize their reproductive success, suggests that prolactin secretion flexibility is adaptive.

Conclusion

My thesis demonstrated that changes in testosterone levels play an important role in physiological, morphological and behavioural differences between males of two different ARTs in African striped mice (chapter 2; 3), but that the decision to disperse and become solitary-living males relies on other factors, e.g. cues from mating partners (chapter 3). Alloparental care was not influenced by testosterone (chapter 1; 2) suggesting that changes in alloparental care after the tactic switch are not caused by testosterone itself. I finally showed that the role of environmental factors (e.g. food availability) is crucial in hormonal flexibility (prolactin levels) (chapter 4). Thus, studies from the African striped mouse suggest a complex relationship between hormonal and environmental factors in the regulation of ARTs (Schradin et al. 2012c). For future studies, I suggest to integrate environmental factors in the behavioural endocrinology approach to understand the proximate mechanisms of ARTs by following this step by step procedure: 1) identification of the signals causing tactic switch, for instance in my thesis, I suggested olfactory cues from mating partners. 2) which environmentally-activated neural pathways are at play in the tactic switch, for instance in my thesis, I suggested the dopamine mesolimbic pathway involved in sexual motivation. 3) finally, whether these environmentally-activated neural pathways are modulated by testosterone, i.e. whether the absence of testosterone signal (e.g. via gonadectomy) decrease the probability of tactic switch and vice versa.

References

Aikey, J. L., Nyby, J. G., Anmuth, D. M. & James, P. J. 2002. Testosterone rapidly reduces anxiety in male house mice (Mus musculus). *Hormones and Behavior*, **42**, 448-460.

Balfour, M. E., Yu, L. & Coolen, L. M. 2004. Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. *Neuropsychopharmacology*, **29**, 718-730.

Ball, G. F. & Balthazart, J. 2011. Sexual arousal, is it for mammals only? *Hormones and Behavior*, **59**, 645-655.

Bancroft, J. 2005. The endocrinology of sexual arousal. *Journal of Endocrinology*, **186**, 411-427.

Been, L. E. & Petrulis, A. 2011. Chemosensory and hormone information are relayed directly between the medial amygdala, posterior bed nucleus of the stria terminalis, and medial preoptic area in male Syrian hamsters. *Hormones and Behavior*, **59**, 536-548.

Bennett, L. N. & Pillay, N. 2001. Responses of male Rhabdomys pumilio to urine of females in different reproductive states. *Proc. 8th Int. Symp. African Small Mammals*, 321–330.

Boitani, C., Chen, C. L. C., Margioris, A. N., Gerendai, I., Morris, P. L. & Bardin, C. W. 1985. Pro-opiomelanocortin-derived peptides in testis - evidence for a possible role in leydig and sertoli-cell function. *Medical Biology*, **63**, 251-258.

Brennan, P. A. & Zufall, F. 2006. Pheromonal communication in vertebrates. *Nature*, **444**, 308-315.

Brennan, P. A. & Kendrick, K. M. 2006. Mammalian social odours: attraction and individual recognition. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **361**, 2061-2078.

Brennan, P. A. & Keverne, E. B. 2004. Something in the air? New insights into mammalian pheromones. *Current Biology*, **14**, R81-R89.

Burmeister, S. S. & Wilczynski, W. 2005. Social signals regulate gonadotropin-releasing hormone neurons in the green treefrog. *Brain Behavior and Evolution*, **65**, 26-32.

Cameron, J. L. & Nosbisch, C. 1991. Suppression of pulsatile lutheinizing-hormone and testosterone secretion during short-term food restriction in the adult male rhesus-monkey (*Macaca mulatta*). *Endocrinology*, **128**, 1532-1540.

Canoine, V., Fusani, L., Schlinger, B. A. & Hau, M. 2003. Brain sensitivity to sex steroids changes across seasons in a tropical bird. *Hormones and Behavior*, **44**, 40-41.

Clutton-Brock, T. H., Russell, A. F., Sharpe, L. L., Young, A. J., Balmforth, Z. & McIlrath, G. M. 2002. Evolution and development of sex differences in cooperative behavior in meerkats. *Science*, **297**, 253-256.

Cockburn, A. 1998. Evolution of helping behavior in cooperatively breeding birds. *Annual Review of Ecology and Systematics*, **29**, 141-177.

Dawson, A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 1621-1633.

Dominguez, J. M. & Hull, E. M. 2005. Dopamine, the medial preoptic area, and male sexual behavior. *Physiology & Behavior*, **86**, 356-368.

Eikenaar, C., Whitham, M., Komdeur, J., van der Velde, M. & Moore, I. T. 2011. Endogenous testosterone is not associated with the trade-off between paternal and mating effort. *Behavioral Ecology*, **22**, 601-608.

Endo, D. & Park, M. K. 2004. Molecular characterization of the leopard gecko POMC gene and expressional change in the testis by acclimation to low temperature and with a short photoperiod. *General and Comparative Endocrinology*, **138**, 70-77.

Finch, C. E. & Rose, M. R. 1995. Hormones and the physiological architecture of life-history evolution. *Quarterly Review of Biology*, **70**, 1-52.

Forlano, P. M. & Bass, A. H. 2011. Neural and hormonal mechanisms of reproductive-related arousal in fishes. *Hormones and Behavior*, **59**, 616-629.

Fusani, L. & Hutchison, J. B. 2003. Lack of changes in the courtship behaviour of male ring doves after testosterone treatment. *Ethology Ecology & Evolution*, **15**, 143-157.

Gerendai, I., Shaha, C., Thau, R. & Bardin, C. W. 1984. Do testicular opiates regulate leydig-cell function. *Endocrinology*, **115**, 1645-1647.

Gleason, E. D., Fuxjager, M. J., Oyegbile, T. O. & Marler, C. A. 2009. Testosterone release and social context: When it occurs and why. *Frontiers in Neuroendocrinology*, **30**, 460-469.

Hau, M., Wikelski, M., Gwinner, H. & Gwinner, E. 2004. Timing of reproduction in a Darwin's finch: temporal opportunism under spatial constraints. *Oikos*, **106**, 489-500.

Hews, D. K. & Moore, M. C. 1996. A critical period for the organization of alternative male phenotypes of tree lizards by exogenous testosterone? *Physiology & Behavior*, **60**, 425-429.

Hews, D. K., Knapp, R. & Moore, M. C. 1994. Early exposure to androgens affects adult expression of alternative male types in tree lizards. *Hormones and Behavior*, **28**, 96-115.

Hull, E. M. 1995. Dopaminergic influences on male rat sexual behavior. In: *Neurobiological effects of sex steroid hormones* (Ed. by P. E. Micevych & H. R.P.), pp. 234-253. Cambridge: Cambridge University Press.

Hull, E. M. & Dominguez, J. M. 2007. Sexual behavior in male rodents. *Hormones and Behavior*, **52**, 45-55.

Hull, E. M., Lorrain, D. S., Du, J. F., Matuszewich, L., Lumley, L. A., Putnam, S. K. & Moses, J. 1999. Hormone-neurotransmitter interactions in the control of sexual behavior. *Behavioural Brain Research*, **105**, 105-116.

Jackson, C. & Bernard, R. T. F. 2005. Effects of supplementary food on the winter inhibition of reproduction in male and female four-striped field mice (Rhabdomys pumilio). *Reproduction Fertility and Development*, **17**, 393-400.

Johnston, R. E. & Bronson, F. 1982. Endocrine control of female mouse odors that elicit lutheinizing-hormone surges and attraction in males. *Biology of Reproduction*, **27**, 1174-1180.

Johnstone, R. A. & Cant, M. A. 2008. Sex differences in dispersal and the evolution of helping and harming. *American Naturalist*, **172**, 318-330.

Karsch, F. J., Bittman, E. L., Foster, D. L., Goodman, R. L., Legan, S. J. & Robinson, J. E. 1984. NEUROENDOCRINE BASIS OF SEASONAL REPRODUCTION. *Recent Progress in Hormone Research*, **40**, 185-232.

Keverne, E. B. 2004. Importance of olfactory and vomeronasal systems for male sexual function. *Physiology & Behavior*, **83**, 177-187.

Kleitz-Nelson, H. K., Dominguez, J. M. & Ball, G. F. 2010a. Dopamine Release in the Medial Preoptic Area is Related to Hormonal Action and Sexual Motivation. *Behavioral Neuroscience*, **124**, 773-779.

Kleitz-Nelson, H. K., Dominguez, J. M., Cornil, C. A. & Ball, G. F. 2010b. Is Sexual Motivational State Linked to Dopamine Release in the Medial Preoptic Area? *Behavioral Neuroscience*, **124**, 300-304.

Knapp, R., Hews, D. K., Thompson, C. W., Ray, L. E. & Moore, M. C. 2003. Environmental and endocrine correlates of tactic switching by nonterritorial male tree lizards (Urosaurus ornatus). *Hormones and Behavior*, **43**, 83-92.

Lynn, S. E. 2008. Behavioral insensitivity to testosterone: Why and how does testosterone alter paternal and aggressive behavior in some avian species but not others? *General and Comparative Endocrinology*, **157**, 233-240.

Lynn, S. E. & Wingfield, J. C. 2008. Dissociation of testosterone and aggressive behavior during the breeding season in male chestnut-collared longspurs, Calcarius ornatus. *General and Comparative Endocrinology*, **156**, 181-189.

Lynn, S. E., Hayward, L. S., Benowitz-Fredericks, Z. M. & Wingfield, J. C. 2002. Behavioural insensitivity to supplementary testosterone during the parental phase in the chestnut-collared longspur, - Calcarius ornatus. *Animal Behaviour*, **63**, 795-803.

Meredith, M. 1998. Vomeronasal, olfactory, hormonal convergence in the brain - Cooperation or coincidence? In: *Olfaction and Taste Xii: An International Symposium* (Ed. by C. Murphy), pp. 349-361.

Moore, M. C. 1991. Application of organizational activation theory to alternative male reproductive strategies - a review. *Hormones and Behavior*, **25**, 154-179.

Moore, M. C., Hews, D. K. & Knapp, R. 1998. Hormonal control and evolution of alternative male phenotypes: Generalizations of models for sexual differentiation. *American Zoologist*, **38**, 133-151.

Nelson, R. J., Gubernick, D. J. & Blom, J. M. C. 1995. Influence of photoperiod, green food, and water availability on reproduction in male california mice (peromyscus-californicus). *Physiology & Behavior*, **57**, 1175-1180.

Oliveira, R. F., Canário, A. V. M. & Ros, A. F. H. 2008. Hormones and alternative reproductive tactics in vertebrates. In: *Alternative Reproductive Tactics* (Ed. by R. F. Oliveira, M. Taborsky & H. J. Brockmann), pp. 132-173. Cambridge: Cambridge University Press.

Pelletier, J., Garnier, D. H., Dereviers, M. M., Terqui, M. & Ortavant, R. 1982. Seasonal-variation in LH and testosterone release in rams of 2 breeds. *Journal of Reproduction and Fertility*, **64**, 341-346.

Phoenix, C. H., Goy, R. W., Gerall, A. A. & Young, W. C. 1959. Organizing action of prenatally administrated tetosterone propionate on the tissues mediating mating behaviour in the female guinea pig. *Endocrinology*, **65**, 369-382.

Piersma, T. & Drent, J. 2003. Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology & Evolution*, **18**, 228-233.

Rivier, C. & Rivest, S. 1991. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis - peripheral and central mechanisms. *Biology of Reproduction*, **45**, 523-532.

Roberts, R. L., Miller, A. K., Taymans, S. E. & Carter, C. S. 1998. Role of social and endocrine factors in alloparental behavior of prairie voles (Microtus ochrogaster). *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **76**, 1862-1868.

Roberts, R. L., Zullo, A., Gustafson, E. A. & Carter, C. S. 1996. Perinatal steroid treatments alter alloparental and affiliative behavior in prairie voles. *Hormones and Behavior*, **30**, 576-582.

Schoepf, I. & Schradin, C. 2012a. Better off alone! Reproductive competition and ecological constraints determine sociality in the African striped mouse (Rhabdomys pumilio). *Journal of Animal Ecology*, **81**, 649-656.

Schoepf, I. & Schradin, C. 2012b. Flexibility in social behaviour and predispositions to change reproductive tactics in African striped mice (Rhabdomys pumilio). *Animal Behaviour*, **84**, 1159-1167.

Schradin, C. 2008a. Seasonal changes in testosterone and corticosterone levels in four social classes of a desert dwelling sociable rodent. *Hormones and Behavior*, **53**, 573-579.

Schradin, C. 2008b. Differences in prolactin levels between three alternative male reproductive tactics in striped mice (Rhabdomys pumilio). *Proceedings of the Royal Society B-Biological Sciences*, **275**, 1047-1052.

Schradin, C. & Lindholm, A. K. 2011. Relative fitness of alternative male reproductive tactics in a mammal varies between years. *Journal of Animal Ecology*, **80**, 908-917.

Schradin, C. & Yuen, C.-H. 2011. Hormone levels of male African striped mice change as they switch between alternative reproductive tactics. *Hormones and Behavior*, **60**, 676-680.

Schradin, C. & Pillay, N. 2006. Female striped mice (Rhabdomys pumilio) change their home ranges in response to seasonal variation in food availability. *Behavioral Ecology*, **17**, 452-458.

Schradin, C., Eder, S. & Müller, K. 2012a. Differential investment into testes and sperm production in alternative male reproductive tactics of the African striped mouse (Rhabdomys pumilio). *Hormones and Behavior*, **61**, 686-695.

Schradin, C., König, B. & Pillay, N. 2010a. Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515-521.

Schradin, C., Koenig, B. & Pillay, N. 2010b. Reproductive competition favours solitary living while ecological constraints impose group-living in African striped mice. *Journal of Animal Ecology*, **79**, 515-521.

Schradin, C., Schneider, C. & Yuen, C. H. 2009a. Age at puberty in male African striped mice: the impact of food, population density and the presence of the father. *Functional Ecology*, **23**, 1004-1013.

Schradin, C., Scantlebury, M., Pillay, N. & Koenig, B. 2009b. Testosterone Levels in Dominant Sociable Males Are Lower than in Solitary Roamers: Physiological Differences between Three Male Reproductive Tactics in a Sociably Flexible Mammal. *American Naturalist*, **173**, 376-388.

Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., König, B. & Pillay, N. 2012b. Social flexibility and social evolution in mammals: a case study of the African striped mouse (Rhabdomys pumilio). *Molecular Ecology*, **21**, 541-553.

Schradin, C., Lindholm, A. K., Johannesen, J., Schoepf, I., Yuen, C.-H., Koenig, B. & Pillay, N. 2012c. Social flexibility and social evolution in mammals: a case study of the African striped mouse (Rhabdomys pumilio). *Molecular Ecology*, **21**, 541-553.

Sharp, P. J. 2005. Photoperiodic regulation of seasonal breeding in birds. In: *Trends in Comparative Endocrinology and Neurobiology* (Ed. by H. Vaudry, E. Roubos, L. Schoofs, G. Fiik & D. Larhammar), pp. 189-199.

Silverin, B., Baillien, M. & Balthazart, J. 2004. Territorial aggression, circulating levels of testosterone, and brain aromatase activity in free-living pied flycatchers. *Hormones and Behavior*, **45**, 225-234.

Simerly, R. B. 1995. Hormonal regulation of limbic and hypothalamic pathways. In: *Neurobiological effects of sex steroid hormones* (Ed. by P. E. Micevych & H. R.P.), pp. 85-114. Cambridge: Cambridge University Press.

Sinervo, B. & Svensson, E. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos*, **83**, 432-442.

Solmsen, N., Johannesen, J. & Schradin, C. 2011. Highly asymmetric fine-scale genetic structure between sexes of African striped mice and indication for condition dependent alternative male dispersal tactics. *Molecular Ecology*, **20**, 1624-1634.

Taborsky, M., Oliveira, R. F. & Brockmann, H. J. 2008. The evolution of alternative reproductive tactics: concepts and questions. In: *Alternative Reproductive Tactics: An Integrative Approach* (Ed. by R. F. Oliveira, M. Taborsky & H. J. Brockmann), pp. 1-21. Cambridge: Cambridge University Press.

Tena-Sempere, M., Pinilla, L., Gonzalez, L. C., Dieguez, C., Casanueva, F. F. & Aguilar, E. 1999. Leptin inhibits testosterone secretion from adult rat testis in vitro. *Journal of Endocrinology*, **161**, 211-218.

Wade, J. 2011. Relationships among hormones, brain and motivated behaviors in lizards. *Hormones and Behavior*, **59**, 637-644.

Wikelski, M., Steiger, S. S., Gall, B. & Nelson, K. N. 2005. Sex, drugs and mating role: testosterone-induced phenotype-switching in Galapagos marine iguanas. *Behavioral Ecology*, **16**, 260-268.

Wilczynski, W., Lynch, K. S. & O'Bryant, E. L. 2005. Current research in amphibians: Studies integrating endocrinology, behavior, and neurobiology. *Hormones and Behavior*, **48**, 440-450.

Wilczynski, W., Allison, J. D. & Marler, C. A. 1993. Sensory pathways linking social and environmental cues to endocrine control regions of amphibian forebrains. *Brain Behavior and Evolution*, **42**, 252-264.

Wood, R. I. & Coolen, L. M. 1997. Integration of chemosensory and hormonal cues is essential for sexual behaviour in the male Syrian hamster: Role of the medial amygdaloid nucleus. *Neuroscience*, **78**, 1027-1035.

Wood, R. I. & Newman, S. W. 1995. Hormonal influence on neurons of the mating behavior pathway in male hamsters. In: *Neurobiological effects of sex steroid hormones* (Ed. by P. E. Micevych & H. R.P.), pp. 3-39. Cambridge: Cambridge University Press.

Yahr, P. 1995. Neural circuitry for the hormonal control of male sexual behavior. In: *Neurobiological effects of sex steroid hormones* (Ed. by P. E. Micevych & H. R.P.), pp. 40-56. Cambridge: Cambridge University Press.

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Albert Einstein

"If everyone is moving forward together, then success takes care of itself."

Henry Ford

"A man should never neglect his family for business."

Walt Disney

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Raynaud J, Schradin C (in press). Regulation of male prolactin levels in an opportunistic breeding species, the African striped mouse. Journal of Zoology.

Raynaud J, Schradin C (under review). Experimental increase of testosterone increases boldness and decreases anxiety in male African striped mouse helpers. Physiology & Behavior.

Raynaud J, Müller K, Schradin C (2012). Experimental increase of testosterone levels in free-ranging juvenile male African striped mice (Rhabdomys pumilio) induces physiological, morphological, and behavioral changes. General and Comparative Endocrinology, 178: 108-115.
DOI: 10.1016/j.ygcen.2012.04.028

Raynaud J, Messaoudi F, Gouat P (2012). Reliability of odour-genes covariance despite diet changes: a test in mound-building mice. Biological Journal of the Linnean Society, 106: 682-688.
DOI: 10.1111/j.1095-8312.2012.01888.x

Raynaud J, Dobson SF (2011). Scent communication by female Columbian ground squirrels, *Urocitellus columbianus*. Behavioral Ecology and Sociobiology, 65: 351–358.
DOI: 10.1007/s00265-010-1052-7